University of Alberta

Lung and Airway Cell Composition in Endurance Trained and Untrained Subjects Following an Acute Maximal Exercise Bout

by

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Abstract

This study assessed the effect of a 5 km cycling time trial (TT) on airway/pulmonary cell composition using sputum induction in untrained (UT) and endurance trained (ET) men. ET athletes also performed another TT matched in intensity to that of the UT group (TT-MOD). ET athletes had a higher $\dot{V}O_2$ peak, completed the TT faster, maintained a higher $\dot{V}O_2$ but not V_E and at a higher absolute intensity compared to UT ($P<0.05$). ET athletes had significantly higher % neutrophil at rest than UT subjects. No significant changes in red blood cells were observed 2-hrs after the TT in either group. Both groups showed increased bronchial epithelial cells (BEC) 2-hrs after the TT indicating possible shear stress on the airways. ET athletes had lower BECs following the TT-MOD ($P<0.05$) compared to the UT group following their TT. In conclusion, intense acute exercise can stimulate a change in some but not all lung/airway cells and ET athletes presented with elevated neutrophils at rest and showed fewer changes in lung and airway cell composition than UT subjects at the same absolute intensity.

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Nomenclature

BGB	Blood-gas Barrier
PO_2	Partial Pressure of Oxygen
$AaDO_2$	Alveolar-Arterial PO_2 difference
BAL	Bronchoalveolar lavage
PAP	Pulmonary Artery Pressure
PAWP	Pulmonary Artery Wedge Pressure
EIH	Exercise-Induced Hypoxemia
V_A/Q	Ventilation-Perfusion
Q	Cardiac Output
ECP	Eosinophil Cationic Protein
EIB	Exercise-Induced Bronchospasm
BHR	Bronchial Hyperresponsiveness
BEC	Bronchial Epithelial Cells
NO	Nitric Oxide
PMN	Polymorphonuclear neutrophils

Chapter 1

1.1 Introduction

The pulmonary blood-gas barrier is an extraordinary structure because of its extreme thinness, immense integrity, and enormous area (West, 2000). While it must be extremely thin to decrease the resistance to gas exchange, it also must be robust enough to withstand increased capillary wall pressures which develop when capillary pressure increases with exercise (Wagner, 1986). The increase in pulmonary artery pressure (PAP) during strenuous exercise is beneficial because it facilitates capillary recruitment and an increase in diffusion. However, the resulting increase in pulmonary capillary pressure with exercise may be excessive and lead to exercise-induced pulmonary damage (West, 2000). It is possible the high pulmonary vascular pressures that accompany exercise may be damaging to the delicate pulmonary capillaries. While some human evidence of ruptured pulmonary capillaries during exercise exists, the nature and extent of this damage is still disputed (Edwards, 2000; Eldridge, 2006; Hanel, 2003; Hopkins, 1997; Morici 2004).

As pulmonary artery pressure rises, fluid moves from the capillary lumen into the alveolar wall interstitium and possibly into the alveolar space (West, 2004). Thus, this induced pulmonary edema would become more severe as PAP increases and as a direct result of high capillary transmural pressures, continued increases in PAP may cause pulmonary rupture or damage to the capillary endothelial and alveolar epithelial cell layers (West, 2004). Hopkins et al. (1997) found increased concentrations of red blood cells and proteins in bronchoalveolar lavage fluid of highly trained athletes, and concluded that this was evidence of damage to the blood gas barrier. As a result of this latter research, it has been assumed that athletes are exposed to greater pulmonary

vascular pressures during exercise because of their greater cardiac output and, therefore, would be more likely to develop (greater) edema/damage/inflammation. Importantly, direct measurement of pulmonary vascular pressures during exercise has shown that pulmonary venous pressure was actually lower in endurance trained athletes during exercise as compared to sedentary subjects (Stickland, 2006a). It was postulated that the adaptations that allow an endurance trained athlete to achieve a higher cardiac output may also prevent the development of exaggerated pulmonary vascular pressures during exercise (Stickland, 2006b). Levine et al. (1991) used lower body negative pressure and saline infusion to demonstrate that at the same pulmonary artery wedge pressure, athletes had higher stoke volume (SV) compared with controls. As a result, it was hypothesized by Levine et al. that chronic intense exercise produces a volume overload to the heart which increases ventricular compliance. Stickland et al. (2006b) stated that to achieve greater pulmonary flow rates (Q), well trained subjects accomplished the increased driving pressure not through a higher PAP but rather, by having better cardiac compliance and therefore, lower pulmonary venous pressures compared with less fit subjects. As such, it remains to be determined whether untrained subjects experience greater exercise-induced lung damage as compared to endurance trained subjects when performing maximal exercise.

In addition to pulmonary damage, it has been speculated that airway inflammation can occur as a result of high ventilation during exercise (Bonsignore, 2001; Bonsignore, 2003). Wetter et al. (2001) suggested that airway inflammation could lead to airway edema, excessive mucus production, and smooth muscle constriction in the small airways, resulting in a reduced distribution of alveolar ventilation during exercise.

Moreover, acute and chronic endurance exercise training has been shown to cause airway inflammation in animal and human models (Chimenti et al, 2007; Bonsignore et al, 2003). Training for 45 days caused progressive damage, apoptosis, and proliferation of bronchial epithelium in mice at rest (Chimenti et al, 2007). However, it was concluded in this latter study that the increased apoptosis of bronchial epithelial cells (BECs) after training was a reflection of damage rather than a mechanism limiting inflammatory activation and that habitual exercise at increasing loads may increase epithelial turnover in bronchioles. In humans, increased neutrophils, eosinophils, and lymphocytes alone or in combination were reported in the airways of runners (Bonsignore, 2001), swimmers (Bonsignore, 2003 and Helenius, 1998), and skiers (Karjalainen, 2000). Bonsignore et al. (2001) found high polymorphonuclear neutrophil counts in induced sputum of five middle aged, amateur runners following a marathon. In addition, Morici et al (2004) collected induced sputum samples in a group of young competitive rowers at rest and within 1 hour after a short all-out rowing test over 1000m (about 3min) and found that post-exercise total corrected cell counts were highest in athletes showing the highest minute ventilation per kilogram and tidal volume per kilogram during exercise. This finding supported the possibility that high airflows during all-out exercise may cause epithelial damage through shear stress on the airway wall. It has been speculated that the mechanism(s) responsible for the increased bronchial epithelial cells was epithelial shear stress that can influence the structure, function, and metabolism of lung cells and lead to lung damage (Chimenti et al, 2007; Morici et al, 2004). It is important to note that most previous studies obtained airway cell samples in athletes at rest and not post-exercise. More recently, the effects of acute exercise on airway cells have been analyzed. Increased

or unchanged neutrophil counts compared with baseline have been documented in non-asthmatic endurance athletes after competitions (Bonsignore et al, 2001; Bonsignore et al, 2003). Unfortunately, limited data are available in human subjects on the relationship between exercise intensity and fitness level on post-exercise airway cell counts or composition.

1.2 Purpose and Hypothesis

The purpose of this study was to examine the composition of airway and lung cells using simple sputum induction at rest and following acute maximal exercise in endurance trained (ET) and non-aerobically trained (UT) male participants. It was hypothesized that endurance trained subjects would have little to no red blood cells present (indicating damage to the pulmonary blood gas barrier) and increased presence of inflammatory cell markers (neutrophils and bronchial epithelial cells) in their sputum sample following maximal exercise. In addition, at the same average absolute power output, it was hypothesized that endurance trained men would have fewer red blood cells and fewer markers of airway inflammation (as above) present in their sputum than untrained men.

1.3 Significance

The topic of exercise-induced lung damage remains an area of controversy and considerable debate (Hopkins et al, 1994; Hopkins et al, 1997; Levine et al, 1991; Stickland et al, 2006a,b). For years it has been assumed that the lung has been "overbuilt" for exercise (West, 1998). While the currently held belief is that trained subjects can elicit lung damage, findings by Levine et al. (1991) and more recently, Stickland et al. (2006a, b) offer an opposing perspective. To date, no studies have compared sputum cell counts

in aerobically trained and untrained individuals at rest and following exercise. Further investigation of the existence of pulmonary edema/damage/airway inflammation as a consequence of exercise and in individuals of differing fitness levels will allow for a better understanding of the integrity of the pulmonary system during exercise.

1.4 References

1. Bonsignore M, Morici G, Riccobono L, Insalaco G, Bonanno A, Profita M, Paterno A, Vassalle C, Mirabella A, and Vignola A. Airway inflammation in nonasthmatic amateur runners. *Am J Physiol Lung Cell Mol Physiol* 281: L668-676, 2001.
2. Bonsignore M, Morici G, Vignola A, Riccobono L, Bonanno A, Profita M, Abate P, Scichilone N, Amato G, Bellia V, and Bonsignore G. Increased airway inflammatory cells in endurance athletes: what do they mean? *Clinical Exp Allergy* 33:14-21, 2003.
3. Chimenti L, Morici G, Paterno A, Bonanno A, Siena L, Licciardi A, Veca M, Guccione W, Macaluso F, Bonsignore G, and Bonsignore M. Endurance training damages small airway epithelium in mice. *Am J Respir Crit Care Med* 175: 442-449, 2007.
4. Edwards MR, Hunte GS, Belzberg AS, Sheel AW, Worsley DF, and McKenzie DC. Alveolar epithelial integrity in athletes with exercise-induced hypoxemia. *J Appl Physiol* 89: 1537-1542, 2000.
5. Eldridge MW, Braun RK, Yoneda KY, and Walby WF. Effects of altitude and exercise on pulmonary capillary integrity: evidence for subclinical high altitude pulmonary edema. *J Appl Physiol* 100: 972-980, 2006.
6. Hanel B, Law I, and Mortensen J. Maximal rowing has an acute effect on the blood-gas barrier in elite athletes. *J Appl Physiol* 25: 25, 2003.

7. Helenius IJ, Rytila P, Metso T, Haahtela T, Venge P, and Tikkanen HO. Respiratory symptoms, bronchial responsiveness, and cellular characteristics of induced sputum in elite swimmers. *Allergy* 53: 346-352, 1998.
8. Helenius I, Rytila P, Sarna S, Lumme A, Helenius M, Remes V, Haahtela T. Effect of continuing or finishing high-level sports on airway inflammation, bronchial hyperresponsiveness, and asthma: a 5-year prospective follow-up study of 42 highly trained swimmers. *J Allerg Clim Immunol*. Jun;109(6):962-8, 2002.
9. Hopkins SR, Schoene RB, Henderson WR, Spragg RG, Martin TR, and West JB. Intense exercise impairs the integrity of the pulmonary blood-gas barrier in elite athletes. *Am J Respir Crit Care Med* 155: 1090-1094, 1997.
10. Karjalainen E, Laitinen A, Sue-Chu M, Altraja A, Bjermer L, and Laitinen L. Evidence of airway inflammation and remodeling in ski athletes with and without bronchial hyperresponsiveness to methacholine. *Am J Respir Crit Care Med* 161: 2086-2091, 2000.
11. Levine BD, Lane LD, Buckey JC, Friedman DB, and Blomqvist CG. Left ventricular pressure-volume and frank-starling relations in endurance athletes. Implications for orthostatic tolerance and exercise performance. *Circulation* 84: 1016-1023, 1991.
12. Morici G, Bonsignore M, Zangla D, Loredana Riccobono L, Profita M, Bonanno A, Paterno A, Giorgi R, Mirabella F, Chimenti L, Benigno A, Vignola A, Bellia V, Amato G, and Bonsignore G. Airway cell composition at rest and after an all-out test in competitive rowers. *Med Sci Sports Exercise* 36: 1723-1729, 2004.

13. Stickland MK, Welsh RC, Haykowsky MJ, Petersen SR, Anderson WD, Taylor DA, Bouffard M and Jones RL. Effect of acute increases in pulmonary vascular pressures on exercise pulmonary gas exchange. *J Appl Physiol* 100: 1910-1917, 2006a.

14. Stickland MK, Welsh RC, Petersen SR, Tyberg JV, Anderson WD, Jones RL, Taylor DA, and Haykowsky MJ. Does fitness level modulate the cardiovascular hemodynaminc response to exercise? *J Appl Physiol* 100: 1895-1901, 2006b.

15. Wagner PD, Gale GE, Moon RE, MmHgé-Bueno JR, Stolp BW, and Saltzman HA. Pulmonary gas exchange in humans exercising at sea level and simulated altitude. *J Appl Physiol* 61: 260-270, 1986.

16. Wetter TJ, Xiang Z, Sonetti DA, Haverkamp HC, Rice AJ, Abbasi Aa, Meyer KC, Dempsey JA. Role of lung inflammatory mediators as a cause of exercise-induced arterial hypoxemia in young athletes. *J Appl Physiol*. Jul;93(1):116-26, 2002.

17. West JB. Invited Review: pulmonary capillary stress failure. *J Appl Physiol* 89: 2483-2489; discussion 2397, 2000.

18. West JB. Vulnerability of pulmonary capillaries during exercise. *Exerc Sport Sci Rev* 32: 24-30, 2004.

19. West JB, and Wagner PD. Pulmonary gas exchange. *Am J Respir Crit Care Med* 157: S82-87, 1998.

Chapter 2

Literature Review

2.1 The Theory behind Pulmonary Damage

With incremental aerobic exercise there is a progressive translocation of a portion of total blood flow into the thorax (Boushnel et al, 2000). This increases cardiac filling pressures and acts to maintain or increase stroke volume despite a drop in cardiac filling time secondary to an increase in heart rate. The translocation of the blood with exercise will increase pulmonary artery and venous pressure, and therefore pulmonary capillary wall stress. While the increase in pulmonary artery pressure (PAP) may be beneficial for exercise because it causes capillary recruitment and an increase in the pulmonary diffusion, some would suggest that the resulting increase in pulmonary capillary pressure with exercise may be excessive and lead to exercise-induced pulmonary damage (Hopkins et al, 1997; Wagner et al, 1986; West and Costello, 1992; West, 2004).

A thin barrier is most optimal for efficient gas exchange by diffusion. Conversely, a stronger, thicker barrier is required to withstand the increased pulmonary capillary pressure and longitudinal tension in the alveolar wall associated with lung inflation (West, 2004). West (2004) has described how pulmonary edema/damage or even hemorrhage can develop with incremental exercise secondary to high pulmonary vascular pressures. The concept of "stress failure" was introduced after West and Costello (1992) demonstrated disruptions to the capillary endothelium, alveolar epithelium, and their respective basement membranes, in a rabbit lung model when pulmonary artery pressures were raised to >40MmHg (West, 2004). The blood gas barrier (BGB) in humans has a thickness as small as 0.3μm, which is engineered to allow gas exchange to occur as

efficiently as possible. For a capillary transmural pressure of 40mmHg and a wall thickness of 0.3μm the calculated wall stress would be 90kPaN/m². This figure is comparable to the wall-stress in the aorta which is protected by large amounts of collagen and elastin (West, 2004).

In order to maintain cardiac output during heavy exercise, the left ventricle filling pressure and consequently, pulmonary venous and ultimately, pulmonary capillary hydrostatic pressure increases. As such, the BGB must be extremely resistant (West, 2004). As pulmonary artery pressure rises, fluid moves from the capillary lumen into the alveolar wall interstitium and possibly into the alveolar space (West and Costello, 1992). Hypothetically, the induced pulmonary edema would become more severe as PAP increases, and, as a direct result of high capillary transmural pressures, further increases in PAP may cause pulmonary rupture or damage to the capillary endothelial and alveolar epithelial cell layers (West and Costello, 1992).

2.2 Empirical Data Showing Damage

Hopkins et al. (1997) demonstrated the first empirical evidence of exercise-induced lung damage. Six male elite cyclists ("high caliber", no fitness data given) raced up a 4km hill as fast as possible, with the winner receiving a cash bonus of one hundred dollars. Average time for completion was 7 minutes, with entire race intensity averaging 92% age-predicted heart rate. Three sedentary subjects acted as controls and did not race. Bronchoalveolar lavage was performed in all subjects following the race and increased red blood cells, total protein, and leukotriene B4 were found in the exercised subjects. These markers indicate that the permeability of the blood-gas barrier to protein and red blood cells is increased but because no other proteins were observed in the BAL fluid

(circulating antibodies immunoglobin G, and immunoglobin M), it was inferred that the BGB retains a sieving function for large-molecular weight proteins. Leukotriene B4 is indicative of activation of neutrophils or other white cells by exposed basement membrane. The prescence of LTB4 may therefore imply evidence of structural damage to the blood-gas barrier with rupture of the capillary endothelium, basement membrane, and the alveolar epithelium. Based on these results, Hopkins et al. (1997) speculated that high intensity exercise greatly increases pulmonary capillary pressure, altering the integrity of the blood-gas barrier and increasing permeability to red blood cells and proteins. It should be noted however, that all of the participants (n=6) had a history of hemoptysis after exercise and that red blood cells were observed in BAL from one sedentary subject. Hopkins further pursued this idea in a follow-up study and subsequently did not find evidence of damage to the BGB following one hour of submaximal (77% VO2max) cycling (Hopkins et al, 1998). These findings are continually cited as the best evidence for exercise-induced lung damage.

Eldridge et al. (1998) studied 5 subjects at altitude and that performed three repetitions of cycle ergometry at 85% VO_2max for five minutes with a 5 minute recovery at 30% VO_2max in between. Bronchoalveolar lavages were performed 2 hours and 24 hours post exercise and inflammatory markers (red blood cells, white blood cells, total cell count) were found within the lavage fluid, suggesting epithelial injury. Unfortunately, non-exercise controls were not used, making it difficult to discern whether the damage was due to altitude, the technique, or the exercise stress itself. In a follow-up study, Eldridge et al. (2006) found that heavy exercise during both normobaric and hypobaric hypoxia resulted in significantly greater leakage of red blood cells and protein

into the alveolar space. Red blood cell counts in the bronchoalveolar lavage fluid remained elevated above baseline non-exercising values but were significantly lower than that found 2 hours post-exercise. In addition, with recovery at 3810m altitude, both alveolar red blood cell and protein concentrations were significantly higher than in the 2 hour post-exercise and non-baseline exercising values. Eldridge speculated that this delay in red blood cell occurrence reflected a slower alveolar clearance of red blood cells via phagocytosis rather than ongoing capillary leakage (2006). These findings further support the hypothesis that loss of pulmonary capillary integrity occurs with intense exercise in healthy humans. Furthermore, Eldridge suggested that the mechanism for the capillary leak may be congruent with previous research (Eldridge et al, 1998; Hanel et al, 2003; Hopkins et al, 1997; Wagner et al, 1986) and as such, is related to high pulmonary vascular pressures and regional over-perfusion that occurs during short-duration, high intensity exercise.

2.3 Pulmonary Edema

The studies of Hopkins et al. (1997) and Eldridge et al. (1998, 2006) provide the most compelling evidence for exercise-induced lung damage. Direct evidence of pulmonary edema from exercise in humans is extremely rare and has only been demonstrated in isolated incidences. Young et al. (1987) reported neurogenic pulmonary edema in a healthy untrained subject following a marathon. MacKechnie et al. (1979) described two cases of pulmonary edema in highly trained marathon runners who had just competed in an ultramarathon (90km). Importantly, exercise induced pulmonary edema likely presents a mild 'sub-clinical' form of edema (Stickland, 2004) whereby gas exchange is potentially impaired, but full alveolar flooding and froth development in the

airways is unlikely. Since it is difficult to measure 'sub-clinical' pulmonary edema, indirect methods have been used to infer pulmonary edema following exercise. Increased lung density following a triathlon in highly trained subjects has been ascribed as subclinical pulmonary edema (Caillaud et al, 1995). Hanel et al. (2003) found increased clearing rates, an indication of alveolar epithelial damage, immediately following a 2000m simulated rowing race which was attributed to exercise-induced edema/damage. Clearance rates were further elevated through 20 minutes post-exercise before returning towards baseline 125-132 minutes after exercise. This finding was in contrast to a previous study which did not find increased clearing rates (Edwards et al, 2000), and in response, Hanel et al. (2003) suggested that this discrepancy was due to a considerable time delay (38 minutes after exercise) in obtaining the samples following exercise by Edwards.

2.4 Indirect Evidence – Gas Exchange

With incremental exercise there is a progressive impairment in pulmonary gas exchange as evaluated by the alveolar to arterial PO_2 difference ($AaDO_2$). The magnitude of the $AaDO_2$ appears to be most severe in well-trained endurance athletes exercising at near-maximal intensities. Wagner et al. (1986) investigated the diffusion limitation, ventilation/perfusion inequality (V_A/Q) and their relationship to cardiac output (Q), pulmonary artery pressure (PAP), and pulmonary artery-wedge pressure (PAWP -which reflects pulmonary venous and mean left atrial pressure). Eight subjects were studied, five cyclists and three who were considered sedentary. Subjects rode at sea level and simulated altitude, with peak a power output of 240W (mean VO2 = 3.7L/min, Q= 23.9L, PAP= 37.2mmHg). Mean pulmonary artery pressure rose to >40mmHg at the heaviest

exercise levels at 15000ft. Pulmonary capillary wedge pressures were recorded and reached 20mmHg during heavy exercise at altitude. At peak normoxic exercise Q was 23.9L/min, while mean PAP and PAWP were 37.2mmHg and 21.1mmHg respectively. As pulmonary capillary pressure is difficult to measure in humans, it is estimated to be at least midway between arterial and wedge pressure (West, 2004). Therefore, if PAP and PAWP are 37.2mmHg and 21.1mmHg respectively, during heavy exercise pulmonary capillary pressure should be approximately 29mmHg with capillary pressure at the base of the lung exceeding 35mmHg (West, 2000). With such high vascular pressures, Wagner and colleagues concluded it was highly likely that transcapillary fluid movement was sufficient enough to result in interstitial perivascular and peribronchial fluid accumulation sufficient enough to affect the distributions of ventilation and blood flow (1986). The correlation between PAP and indexes of V_A/Q mismatch supported the idea that the higher the PAP, the higher would be the expected rate of transcapillary fluid movement. Unfortunately, highly trained athletes were not used for this study. These observations have lead to speculation that the increased alveolar-arterial PO_2 difference ($AaDO_2$) typically observed in healthy subjects during exercise is the result of pulmonary edema or lung damage (Edwards et al, 2000; Eldridge et al, 1998; Eldridge et al, 2006; Hanel et al, 2003).

2.5 Relationship between Empirical Evidence and Indirect Evidence

Research has provided evidence that exercise can produce pulmonary damage. While many have suggested that this damage/edema might contribute to the impairment in gas exchange typically observed with exercise, this has not been adequately proven. Edwards et al. (2000) examined pulmonary clearance rates and gas exchange following

incremental exercise to exhaustion. Clearance rates were not increased following graded exercise and were not related to $AaDO_2$. Edwards et al. (2000) argued that exercise of a longer duration caused a further increase in PAP and edema/damage and therefore, a greater subsequent increase in $AaDO_2$. However it is possible that edema developed rapidly at the start of exercise, but fluid balance reached an elevated steady state such that gas exchange was not further impaired (Stickland, 2004).

More recently Stickland et al. (2006a) acutely increased pulmonary vascular pressures during exercise with lower-body positive pressure in an attempt to augment pulmonary damage/edema. Despite an estimated 25% increase in capillary wall-stress, $AaDO_2$ was not increased. This data suggests that there is no direct relationship between pulmonary vascular pressures and $AaDO_2$. Moreover, it is important to note that an increased $AaDO_2$ can be explained by a number of mechanisms: V_A/Q (Hopkins et al, 1994), diffusion limitation (Hopkins et al, 1994; Wagner et al, 1986), and intrapulmonary shunt (Stickland et al. 2004, 2006). Investigations by Wagner (1986) and Hopkins (1994) illustrate that V_A/Q mismatch is responsible for up to 60% of the $AaDO_2$ during incremental exercise with the inequality appearing to be the most prevalent amongst elite athletes during maximal exertion. Both have speculated that the V_A/Q mismatch is secondary to the development of pulmonary edema. The two dominant theories as to the cause of the diffusion limitation are a reduction in pulmonary transit time or an increase in the diffusion distance due to pulmonary edema or lung damage (Hopkins et al, 1996). Finally, shunting refers to blood that enters the arterial system without going through ventilated areas of the lung. Stickland et al. (2004, 2006) suggested that the increase in microvascular pressure, secondary to the rise in cardiac output with incremental exercise,

is the likely mechanism to open intrapulmonary shunt vessels. While $AaDO_2$ may increase with exercise, along with pulmonary vascular pressures, these responses may simply be correlative and not causal.

2.6 Errors in the Logic – Are Athletes at a Higher Risk for Lung Damage/Edema?

Hopkins 1997 (1997) is the most often cited evidence for exercise-induced damage. Many have interpreted this to suggest that athletes are more susceptible to damage than their sedentary colleagues because athletes are able to achieve higher metabolic rates and a higher peak cardiac output. However the adaptations which have allowed an endurance trained athlete to achieve the higher cardiac output also prevent the athlete from developing exaggerated pulmonary vascular pressures during exercise. Levine et al. (1991) demonstrated that Starling curves are shifted upward and to the left at rest in endurance-trained athletes as compared with non-athletes; specifically, at the same pulmonary artery wedge pressure, athletes had elevated stoke volume (SV) compared with controls. As a result, Levine et al. (1991) hypothesized that chronic intense exercise produces a volume overload which increases ventricular compliance and therefore, shifts the Starling curve. To date, only one study has examined whether fitness level modulates the cardiovascular hemodynamic response to exercise. Stickland et al. (2006) examined the effects of fitness level (VO_2max) on the cardiovascular hemodynamic response to incremental exercise in healthy male subjects stratified by fitness level (VO_2max). As previously mentioned, it has been suggested that athletes would have greater pulmonary artery pressure during exercise because of their cardiac output (Q) and, therefore, would be more likely to develop greater edema/damage and a larger impairment in gas exchange. However, Reeves and Taylor (1996) demonstrated that ~86% of the variance

in PAP during upright exercise can be explained by PAWP. Levine et al. (1991) demonstrated that endurance-trained athletes have better left ventricular compliance compared with non-athletes. In other words, at the same pulmonary artery wedge pressure, athletes had elevated stroke volume compared with controls, and at the same stroke volume, PAWP was lower in athletes. In the aforementioned study done by Stickland et al. (2006b), it was found that PAWP at peak exercise was lower in subjects with a high VO_2max, whereas no difference was observed in PAP. These results indicate that, in achieving greater pulmonary flow rates (Q) fitter subjects accomplish the increased driving pressure not through higher PAP but rather, by having better cardiac compliance and, therefore, lower pulmonary venous pressures compared with less fit subjects. As such, it is likely that athletes would have lower pulmonary capillary pressure and decreased edema.

2.7 Conclusion

There is direct evidence that the mechanism for stress failure in pulmonary capillaries is related to high pulmonary vascular pressures and regional over-perfusion which occurs during high intensity exercise (Hopkins et al, 1997; West, 1992; West 2004). In addition, it has been suggested that the higher the PAP, the greater the impairment in gas exchange (Wagner et al, 1986). It remains to be determined whether trained endurance athletes will demonstrate greater pulmonary damage as compared to untrained subjects, as the cardiovascular adaptations observed in trained subjects would act to limit pulmonary vascular pressures during exercise and therefore reduce hydrostatic-induced damage.

2.8 Airway Inflammation: Introduction

The issue of exercise-induced inflammation was initially addressed with regard to the clinical pathogenesis of exercise-induced bronchoconstriction (EIB) (Bonsignore et al, 2003). Exercise-related respiratory symptoms are frequent in athletes, and a low exercise tolerance is common in asthmatic patients (Bonsignore et al, 2003). In asthma, the characteristic changes in the bronchial mucosa are epithelial damage; infiltration of eosinophils, lymphocytes, mast cells, and macrophages; and remodeling of the airway, with thickening of the epithelial basement membrane and enlargement of the bronchial smooth muscle mass (Karjalainen et al, 2000). Increased incidence of asthma in high level athletes and indexes of airway inflammation and remodeling in non-asthmatic athletes suggest that in some individuals, high levels of exercise training may lead to an "asthma-like" condition (Karjalainen et al, 2003; Konig, 1989; Sue-Chu et al, 1999). Subsequent studies in athletes and experimental models, however, underlined that changes in airway cells may occur in athletes independent of symptoms or spirometric changes, raising questions on their pathogenesis and possible consequences (Bonsignore et al, 2001). As a result, the link between EIB and airway inflammation in humans, either healthy or asthmatic, remains elusive.

Airway epithelial and mucosal mast cells, as well as circulating cells that are recruited to the lung, release a variety of inflammatory mediators, including histamine, leukotrienes, prostaglandins, and cytokines (Wetter et al, 2002). These mediators increase microvascular permeability, peripheral airway smooth muscle constriction, mucus secretion, and alter vascular tone and leukocyte activation (Dahlen, 1998). All of these

actions may not only lead to EIB by narrowing small airways but perhaps also contribute to the V_A/Q mismatch and a widened $AaDO_2$ during exercise.

The functional and cellular events triggered by the increased minute ventilation in order to meet the increased metabolic demand of exercise has been studied in experimental models (Freed, 1995; Freed et al, 1999). The main theory behind EIB (a post-exercise decrease in forced expiratory volume of 1 second (FEV_1) of 10% from the baseline value (ATS, 1995) is that the increased minute ventilation with exercise results in drying of the airways, thereby increasing the osmolarity of the airway surface lining (Anderson, 2000). This generated osmotic stimulus makes bronchial epithelial cells shrink and release inflammatory mediators, causing airway smooth muscle contraction or asthma. Experimental hyperventilation with dry and cold air has been shown to cause bronchoconstriction (Freed, 1995) with a major importance of dryness as opposed to cooling. Hyperventilation with dry air caused hyperosmolarity of airway surface lining and bronchoconstriction (Freed et al, 1999). Leukocyte infiltration and epithelial injury have been reported in canine airways within 2h of a 5-min period of hyperventilation with dry air at room temperature, with no evidence of resolution after 24h (Freed et al, 1999). Alternatively, it has been suggested that EIB could reflect decreased airway caliber (damage) secondary to vascular engorgement at airway rewarming following exercise (MacKechnie et al, 1979). Repeated hyperventilation challenges caused epithelial damage with eosinophil and neutrophil influx and increased leukotriene concentrations in bronchial alveolar lavage fluid (Sue-Chu et al, 1999). As such, inflammatory cell recruitment into large airways could be triggered by damage of airway epithelium (Chimenti et al, 2007). Bronchial epithelial cells, in vitro, exposed to

hyperosmolar medium or cooling-rewarming released chemotactic factors, suggesting a role of bronchial epithelial cells in the "proinflammatory" response to exercise (Bonsignore et al, 2001). However, data obtained in vivo tend to be capricious.

2.9 Inflammation in Athletes

The biology of airway cells and inflammatory mediators has been increasingly studied in athletes. Competitive training in athletes is often associated with symptoms and signs of airway inflammation (Bonsignore et al, 2001). Elite athletes of summer (Bonsignore et al, 2001; Bonsignore et al, 2002; Helenius et al, 1998) and winter (Karjalainen et al, 2000; Larson et al, 1993) sports show a high prevalence of exercise-induced respiratory symptoms and spirometric alterations. Skiers and long distance runners regularly expose themselves to lower ambient temperatures for prolonged periods of time and to greater levels of minute ventilation. It has been hypothesized that this more than overwhelms the air-conditioning capacity of the upper airways (Bonsignore et al, 2001). Large airways of athletes exercising in a cold or a moderate environment show evidence of inflammation (Chimenti et al, 2007; Karjalainen et al, 2000). Strong evidence exists relating chronic prolonged exercise to airway inflammation and even permanent damage. Running a marathon increases circulating white blood cells and neutrophil counts as well as plasma levels of pro- and anti-inflammatory cytokines (Bonsignore et al, 2001). In addition, in the days following a race, a high frequency of upper respiratory tract infections were reported by marathon runners, suggesting that intense and prolonged endurance exercise may increase the susceptibility of the respiratory tract to pathogens (Bonsignore et al, 2001). Therefore, the increased risk for EIB in athletes is commonly believed to be linked to exercise hyperventilation (Anderson, 2000), through enhanced

airway exposure to allergens and pollutants in summer sports, and dry and cold air in winter sports. Indeed, 78% of athletes who were EIB-positive after a sport-and environment specific exercise test turned negative when the test was repeated in the laboratory (Rundell et al, 2000). While there may be a relationship between EIB and airway inflammation it should be noted that it is outside the scope of this study.

Studies on airway cells and inflammatory markers in endurance athletes explored the possible association between airway inflammatory cells and exercise-induced respiratory symptoms. Studies differed for sport examined, methodology, assessment of post-exercise changes, and exercise environment (ie. field vs. laboratory). In summary, athletes of endurance sports have showed increased numbers of inflammatory cells in bronchial biopsies, bronchoalveolar lavage fluid, or induced sputum (Bonsignore et al, 2003). Because increased neutrophils were found in all studies (Bonsignore et al, 2003) irrespective of sample type or sport activity, they can be interpreted as possibly secondary to endurance training. Increased eosinophil and lymphocyte counts were found in swimmers (Helenius et al, 1998) and skiers (Karjalainen et al, 2000), respectively, suggesting a major effect of environmental in increasing these cell types.

An important feature of endurance exercise is that it causes a systemic inflammatory response, which includes mobilization and activation of neutrophils and a complex pattern of pro/anti-inflammatory mediator/cytokine release likely related to muscle damage and intense stress (Bonsignore et al, 2003). However, whether exercise affects the airway and systemic compartments similarly remains unexplored.

2.10 Evidence of Chronic Airway Inflammation

Karjalainen et al. (2000) studied forty competitive cross-country skiers from Sweden and Norway, without a prior diagnosis of asthma; 12 mildly asthmatic subjects; and 12 healthy nonathletic control subjects. Fiberoptic bronchoscopy was performed in skiers in the autumn, at the peak of their preseasonal training program. The exact timing of the testing relative to the subject's most recent training was unmentioned. Group analysis showed that skiers had 43-fold ($p<0.001$), 26-fold ($p<0.001$), and twofold ($p<0.001$) greater T-lymphocytes, macrophage, and eosinophil counts, respectively, than did controls. With the exception of T-lymphocytes, skiers had a lesser degree of infiltration with eosinophils, mast cells, and macrophages than steroid naïve subjects with mild asthma. Skiers had greater infiltration of neutrophils which is not a significant feature in either atopic or nonatopic asthma (Bradley et al, 1991) suggesting that the inflammatory process in those athletes is different from that in asthmatic individuals. As a result, Karjalainen et al. (2000) concluded that this was evidence of airway inflammation and remodeling in ski athletes. Furthermore, in contrast to Power et al. (1993), Karjalainen et al. (2000) observed inflammatory changes in both hyperresponsive and nonhyperresponsive skiers. It was suggested by Karjalainen et al. (2000) that it is possible these changes in non-hyperresponsive skiers are related to repeated exposure of the proximal airways to inadequately conditioned air. Lastly, Karjalainen et al. (2000) measured a significantly thicker tenascin-specific immunoreactivity band in the basement membrane of skiers compared to controls (despite being lower than compared to asthmatic subjects) and suggested that this finding may reflect ongoing healing and repair processes and remodeling of the airways. It should be noted however that there were no

strong correlations found between the inflammatory indices and duration of skiing experience or training in skiers.

Airway inflammation in trained subjects showed some anomalies when compared to asthmatic subjects. Firstly, airway inflammation was not always associated with bronchial hyperreactivity or post-exercise respiratory symptoms (Bonsignore et al, 2003; Karjalainen et al, 2000). Secondly, inflammatory cells have not been found to be activated after exercise (Bonsignore et al, 2001) and ski asthma (a syndrome in which strenuous exercise combined with breathing of cold air triggers airway obstruction in subjects with hyperactive airways, Davis et al, 2005) did not respond to inhaled steroids (Bonsignore et al, 2003; Chimenti et al, 2007). It was suggested that training may indeed blunt inflammatory activation in the airways, similar to the effects reported systemically (Chimenti et al, 2007).

2.11 Does Training Cause Airway Inflammation?

Chimenti et al. (2007) tested whether endurance training under standard environmental conditions causes epithelial damage and inflammation in the small airways of mice. Mice were randomly assigned to sedentary (n=14) or trained (n=16) groups and training was performed 5days/week for 6 weeks at progressively increasing loads. At 45 days, body weight was lower in trained than in age matched sedentary mice and muscle hypertrophy, measured via mean fiber diameter of the tibialis anterior, was significantly greater in trained mice. The bronchial epithelium showed progressive changes during training and at 45 days the number of ciliated epithelial cells was fourfold lower in trained compared to sedentary mice, while epithelial thickness was 56% greater and apoptosis of bronchial epithelial cells was almost doubled. In addition, bronchial

epithelium in trained mice showed a fivefold increase in the number of proliferating cells compared to controls, suggesting active repair. While no difference was found between trained and sedentary mice in blood lymphocyte and neutrophil differential counts at any time points, in bronchioles of mice trained for 45 days, leukocyte counts were fivefold higher than in sedentary control animals in both wall and lumen. Chimenti et al. (2007) therefore concluded that training for 45 days caused progressive damage, apoptosis, and proliferation of bronchial epithelium. It was also concluded based on measurement of inflammatory markers (NF-kB) that while endurance training causes epithelial damage and repair, it did not appear to be a powerful proinflammatory stimulus. As a result, increased apoptosis of bronchial epithelial cells (BECs) after training perhaps was a reflection of damage rather than a mechanism limiting inflammatory activation and that habitual exercise at increasing loads may increase epithelial turnover in bronchioles. Overall, the increase Chimenti et al. (2007) observed in the number of inflammatory cells without activation suggested that the observed "inflammation" represented a training adaptation and did not necessarily imply a detrimental effect for respiratory health. Finally, Chimenti et al. (2007) speculated that the mechanism(s) responsible for the changes they observed in endurance trained mice was due to epithelial shear stress; structure, function, and metabolism of lung cells are influenced by physical forces and inappropriate physical forces such as mechanical ventilation in an inhomogeneous lung, may induce lung damage.

2.12 Does Airway Inflammation Limit Exercise?

Wetter et al. (2002) studied the role of inflammatory mediators as a cause of exercise-induced arterial hypoxemia in young athletes. As previously discussed, an excessively wide alveolar-arterial oxygen pressure difference found in elite athletes has been deemed a major contributor to exercise-induced arterial hypoxemia (Demspey and Wagner, 1999). The ventilation perfusion mismatch has also previously been attributed to the possible development of interstitial edema caused by high pulmonary pressures. Prefault et al. (1994) administered nedocromil sodium which prevents mediator release by stabilizing the membranes of mast and (potentially) other cells, to a small group of master athletes (mean age 63 years old) and found that it narrowed the $AaDO_2$ by approximately 50% and attenuated the fall in PaO_2 during incremental exercise. As such, Wetter et al. (2002) suggested that airway inflammation could lead to airway edema, excessive mucus production, and smooth muscle constriction in the small airways, resulting in a poorer distribution of alveolar ventilation during exercise. It was also proposed that increased permeability of the pulmonary or bronchial vasculature could lead to inflammatory influx in airways and stimulate further mediator release which, in turn, contributes to a maldistribution of alveolar ventilation. In both such scenarios, airway inflammation may be a factor in the development of $AaDO_2$. Hopkins et al. found inflammatory mediators in BAL fluid both after strenuous exercise (1997) and in the basal state indicating that both acute and chronic exercise may lead to increased occurrence of inflammatory processes in the lung.

To expand on these findings, Wetter et al. (2002) investigated the role of inflammation in gas-exchange abnormalities and exercise-induced hypoxemia in young

endurance athletes. A histamine-receptor antagonist (fexofenadine), nedocromil sodium, and a leukotriene-synthesis inhibitor (zileuton) were administered together in a one-time dose before exercise. In combination these drugs have been shown to be important in the management of inflammation in asthma (Spooner et al, 2000). Several mediators in plasma, urine, and sputum as well as changes in lung function and respiratory resistance were measured to document the effects on lung inflammation. It was hypothesized that drug administration would reduce inflammatory mediators, improve gas exchange during exercise, and result in less exercise-induced hypoxemia as compared to placebo. Seventeen healthy endurance-trained athletes were selected after screening and administration of interventions was done in a double-blind manner. Subjects performed graded exercise following either pharmacologic intervention or placebo. Blood samples taken during exercise and induced sputum collection were initiated 45 minutes post exercise. It was found that there were no significant effects of drug treatment on ventilatory or metabolic parameters at rest or during submaximal or maximal exercise compared with placebo. The proportion of macrophages, lymphocytes, and neutrophils did not change with exercise relative to baseline, and eosinophils were generally absent from the sputum samples. The only effect found of the drug treatment was to reduce the neutrophil counts in sputum after exercise; however, as a percentage of the white blood cell count, they did not differ from baseline or placebo. Wetter et al. (2002) demonstrated that gas exchange ($AaDO_2$) and SaO_2 in during maximal exercise in young healthy endurance-trained athletes were unaffected by administration of drugs known to reduce or prevent inflammatory responses in the lungs. The relative absence of eosinophils in the samples obtained is consistent with a lack of chronic lung inflammation (Bradley et al,

1991). It should be noted however that sputum induction timing was not standardized and that the exercise protocol was inconsistent with previous methods used by Hopkins et al (1997) to induce airway inflammation/damage. It was therefore concluded that there was little evidence for exercise-induced lung inflammation in young endurance-trained athletes during an incremental graded exercise test. Two possibilities for these results were offered: either the drugs administered did not reduce lung inflammation sufficiently to alter gas exchange in the subjects, or in fit, young athletes, lung inflammation is not a significant contributor to the widened $AaDO_2$ during exercise (Wetter et al, 2002).

2.13 Airway Inflammation in Acute Exercise Bouts

Bonsignore et al. (2001) studied whether running a marathon affects nasal or exhaled nitric oxide (NO), a noninvasive marker of inflammation, in healthy nonasthmatic subjects. Samples of induced sputum and venous blood were collected as well to study airway cells and to explore the relationship between the effects of the race at the systemic airway levels, respectively. This study examined the acute effects of exercise on amateur, middle-aged runners and not merely baseline values to address the chronic effects of training as in the other studies. Nine amateur, non-smoking and clinically healthy male runners were studied after the Fourth Palermo International Marathon on December 8, 1998. Baseline measurements were taken 6-9 weeks following the marathon at the same time of day as in the post-race condition and 24-28h after the last training session. It was found that white blood cell and polymorphonuclear neutrophil (PMN) counts were very high after the marathon, with decreased percentages of lymphocytes and monocytes.

During exercise, the amount of expired NO followed the changes in minute ventilation, with a fall toward baseline in early recovery. This correlates with previous observations of normal exhaled NO values in skiers with "ski asthma" (Larson et al, 1993), competitive swimmers (Helenius et al, 1998) or amateur runners (Bonsignore et al, 2001) tested under baseline conditions. However, about 3h after the marathon, exhaled NO was twice as high as baseline suggesting late changes in the time course of exhaled NO after exercise (Bonsignore et al, 2001). As a result, it was suggested that NO played a possible role for controlling or decreasing airway inflammation after intense prolonged endurance exercise.

Post-exercise exhaled NO may also reflect modulation of bronchial responsiveness (Bonsignore et al, 2001). It has been found that at the same workload, well-trained athletes release more NO than sedentary subjects (Maroun et al, 1995) and increased NO production in athletes has been associated with a higher workload compared to that attained by untrained subjects, suggesting that NO was predominantly affected by exercise intensity rather than by training status (Chirpaz-Oddou et al, 1997). Bonsignore et al. (2001) obtained adequate induced sputum samples only from five subjects after the marathon (202 +/- 28 minutes) and from five subjects at baseline (therefore only from four subjects on both occasions). The cells in the induced sputum from the runners were mostly polymorphonuclear neutrophils (PMNs) as opposed to macrophages in controls. This is congruent with observations of elite skiers (Karjalainen et al, 2000) and elite swimmers (Helenius et al, 1998) however these results were not obtained following an acute exercise bout. In addition, the pattern of adhesion molecules (L-selectin) in the induced sputum indicated a low grade of activation. With this

evidence, Bonsignore et al. (2001) concluded that the high number of PMNs in the induced sputum was not associated with PMN activation systemically. Eosinophil counts in runners were low both at baseline and after the marathon and lymphocyte and bronchial cell counts were similar in the runners and control subjects and in the post-race and baseline samples. Finally, the finding of normal spirometry at a time when markers of airway inflammation were increased suggests a minimal impact of exercise-induced airway changes on airway function and performance of amateur runners during a marathon (Bonsignore et al, 2001). In terms of the mechanism responsible for exercise-induced airway changes in runners, Bonsignore et al. hypothesized that they are linked to hyperventilation for prolonged periods of time (Bonsignore et al, 2001). Repetitive hyperpnea in dogs has been shown to increase eosinophilic inflammation and levels of prostaglandins and leukotrienes in BAL fluid (Freed and Davis, 1999). Mechanical ventilation at high tidal volumes for prolonged periods of time has been shown to cause pulmonary inflammation and cytokine release (Freed, 1995). Excessive mouth breathing and dry air could accentuate the load to humidify the incoming air (Bonsignore et al, 2001). Vascularly, the high pulmonary pressures associated with heavy exercise combined with an exercise-induced rise in circulating inflammatory mediators may increase microvascular permeability (West and Costello, 1992). It should be noted however, that Bonsignore et al. (2001) had difficulty finding relationships due to small sample size.

Rowing was used for the study done by Morici et al. (2004) because it could be used to assess the effects of very intense, short-lived exercise, as it involves a large muscle mass, and causes substantial hyperpnea. Prior to this study, no data was available

in human athletes on the relationship between exercise ventilation and post-exercise airway cell counts or composition. In this study, induced sputum samples were collected in a group of young competitive rowers at rest and after a short all-out rowing test over 1000m (about 3min). Resting samples were obtained a few days before exercise samples and were considered as mostly influenced by chronic effects of habitual training whereas samples obtained after exercise were considered to reflect the acute effects of the exercise bout. It was found that post-exercise total corrected cell counts were highest in athletes showing the highest minute ventilation per kilogram and tidal volume per kilogram during exercise. This effect being accounted for by macrophages and epithelial cells, as neutrophils, eosinophils, and/or lymphocytes did not correlate with any ventilatory variable recorded during exercise. Induced sputum of the rowers at rest was rich in neutrophils, and did not change significantly post-exercise except for a trend toward increased bronchial epithelial cells. This coincides with multiple previous findings (Bonsignore et al, 2001; Chimenti et al, 2007) and lends support for the possibility that high airflows during all-out exercise may cause epithelial damage through shear stress on the airway wall. However, it is impossible to obtain true "resting" samples in athletes on regular intense training, as according to data airway inflammation may still be present 24h post-exercise (Bonsignore et al, 2003).

No change in neutrophil counts in induced sputum or elastase concentration in sputum post-exercise and no evidence of their modulation by the level of exercise V_E was found (Morici et al, 2004). It was suggested that the all-out test was too short in duration to cause neutrophil influx in the airways (Morici et al, 2004). Alternatively, the failure to

observe an increase in neutrophils may have been due to the timing of the induced sputum (within one hour post-exercise) and the time course of neutrophil appearance.

Finally, a decreased expression of L-selectin (an adhesion molecule) by neutrophils and macrophages in induced sputum was observed (Morici et al, 2004). Therefore, it was concluded by Morici et al. that intense exercise may mostly cause acute functional changes in airway cells, and that changes in adhesion molecules only requires a short exercise duration to develop. Morici et al. suggested that the reduction of adhesion molecules could offset the immunostimulatory effects of increased chemotactic expression, possibly accounting for the tight control of inflammatory activation at the airway level in nonasthmatic athletes tested following exercise (Bonsignore et al, 2001).

2.14 Conclusion

There is no question that exercise-related respiratory symptoms are frequent in athletes and may be related to exercise conditions and high minute ventilation (Bonsignore et al, 2003). Evidence of chronic increased airway inflammation has been documented in cross country skiers (Karjalainen et al, 2000), swimmers (Helenius et al, 1998), and marathon runners (Bonsignore et al, 2001). Moreover, recent evidence of increased neutrophils and bronchial epithelial cells in induced sputum following an all-out rowing test supports the idea that acute exercise may cause/increase airway inflammation (Morici et al, 2004). Perhaps the most logical hypothesis concerning airway inflammation is that high airflow rates generated with intense exercise may cause mediator release from lung cells because of shear or mechanical stress or by evaporation-induced changes in osmolality (Freed and Davis, 1999). The stimulus for this observed

inflammation may be related to ventilation or to a 'general' inflammatory response from exercise or both and requires further investigation.

2.15 Induced Sputum

A recent advance in the investigation of airway inflammation has been the introduction of sputum induction by inhalation of an aerosol hypertonic saline (Pin et al, 1992). Induced sputum has a number of advantages over more invasive methods such as bronchoalveolar lavage (BAL) or biopsy. Safety and practicality are the most obvious of these advantages. The invasive techniques are limited in their applicability by discomfort, inconvenience, or risk of bronchoscopy and cannot be easily applied repeatedly (Maestrelli et al, 1995). The method of obtaining induced sputum is relatively noninvasive and can be carried out at random (Pizzichini et al, 2002) and repeatedly in subjects with varying disease severity.

It has been established that sputum induction is a useful noninvasive method for collecting airway secretions from subjects. Analysis of induced sputum for cellular and biochemical constituents has been found to be a valid method for monitoring airway inflammation (Fahy et al, 1995; Gershman et al, 1999; Jatakanon et al, 1998). Studies of sputum cell counts have demonstrated repeatability (Pin et al, 1992), responsiveness (Fahy et al, 1993; Fahy et al, 1995; Maestrelli et al, 1995), and validity (Fahy et al, 1995; Pizzichini et al, 1996; Pizzichini et al, 2002) for determining markers of airway inflammation of the methods. It is a noninvasive and highly reproducible approach in generating a measurable index of inflammatory cells in the airways of the lungs (Lacy et al., 2005).

The validity of induced sputum can be examined by its ability to detect differences between different clinical conditions. Pizzichini et al. (1996) demonstrated differences in induced sputum characteristics among asthmatics, smokers with nonobstructive bronchitis, and healthy subjects. The significant differences observed between subjects with and without asthma symptoms are in keeping with results of BAL studies where symptomatic subjects had higher proportions of eosinophils (markers of airway inflammation). Fahy et al. (1995) found that the percentage of eosinophils and the levels of albumin, fibrinogen, and eosinophil cationic protein (ECP) were higher in induced sputum from asthmatic subjects than in that from healthy subjects. Finally, Lacy et al. (2005) endorsed clinically applying sputum measurements to manage asthmatics.

Maestrelli et al. (1995) examined 21 subjects with bronchial asthma, 12 subjects with stable chronic bronchitis, and 9 subjects with chronic bronchitis during an exacerbation. It was found that there was good agreement between eosinophil counts in sputum, BAL, and bronchial biopsies. It must be noted that in asthma and stable chronic bronchitis, the percentages of neutrophils were significantly higher in sputum than in BAL. The opposite was true of the percentages of macrophages and lymphocytes (Maestrelli et al, 1995). Consequently, it has been suggested that induced sputum measures more peripheral airways than BAL (Keatings et al, 1997; Pizzichini et al, 1996). While Fahy et al. (1995) showed a correlation between induced sputum and BAL, they did conclude that induced sputum samples arise predominantly from airways rather than alveoli. Keatings et al. (1997) as well as Pizzichini et al. (1996) supported this assertion.

However, an unresolved issue in the method for sputum induction is the effect of the duration of sputum induction on cellular and biochemical characteristics of induced sputum. While it could be argued that the induced sputum technique samples the inflammatory cells and soluble markers present in the airway lumen of the bronchial tree, which although reflective of, do not represent an identical situation to the local inflammatory process in the mucous (Kips et al, 2002), recent evidence suggests that increasing the duration of the sputum sampling allows for more peripheral sampling (ie. alveoli sampling) (Holz et al, 1998). Holz et al. (1998) found that neutrophil and eosinophil (airway markers) percentages in induced sputum from healthy and asthmatic subjects decreased during three consecutive 10-minute sputum inductions; in contrast, the percentage of macrophages (alveolar marker) increased in these samples. In addition, Paggiaro et al. (2002) found mucin concentrations to be higher in samples collected early (0-4min) than in samples collected later (16-20min), while surfactant concentrations were higher in samples collected later compared to those collected early. This change in sputum cell count over a longer duration supports that different compartments of the respiratory tract are sampled at different time-points during induction, ie. that central airways are sampled early, while peripheral airways and alveoli are sampled later (Paggiaro et al, 2002).

Gershman et al. (1999) collected induced sputum sequentially at 4 minute intervals during a 20 minute sputum induction in 12 subjects with moderate and mild asthma. Each 4 minute sample was collected and analyzed separately for total and differential cell counts and for levels of ECP, fibrinogen, mucin-like protein, and surfactant SP-A. It was found that the percentages of eosinophils and neutrophils were

significantly higher at the beginning of the 20 minutes sputum induction than at the end. Contrastingly, the percentage of macrophages significantly increased from beginning to end. Furthermore, while the levels of ECP and mucin-like protein were significantly higher at the beginning of the 20 minute induction, the level of surfactant protein SP-A was significantly lower. From this data, they concluded that the duration of sputum induction significantly affects the cellular and biochemical composition of induced sputum in a manner suggesting that large airways are sampled at the beginning of sputum induction, whereas peripheral airways and alveoli are sampled at later time periods.

Finally, the interpretation of the results of induced sputum examination depends on knowledge of normal values from a healthy population. The study done by Belda et al. (2000) is used as the gold standard for reference values for total and differential cell counts in induced sputum from healthy nonsmoking adults. From a total of 118 healthy, nonsmoking adults, all without asthma or airflow obstruction, 96 sputum samples were collected and processed and used to identify a normal range of cell counts in induced sputum. It was demonstrated that the majority of cells are neutrophils and macrophages, whereas eosinophils, lymphocytes, and bronchial epithelial cells are scarce, and metachromatic cells (basophils/mast cells) are almost absent.

2.16 References

1. Anderson SD, Daviskas E. The mechanism of exercise-induced asthma. *J Allergy Clin Immunol* 106: 453-459, 2000.
2. American Thoracic Society: Standardization of spirometry, 1994 update. *Am J Respir Crit Care Med.* 1995;152:1107–36)
3. Belda J, Leigh R, Parameswaran K, O'byrne P, Sears M, and Hargreave E. Induced sputum cell counts in healthy adults. *Am J Respir Crit Care Med* 161:475-478, 2000.
4. Bonsignore M, Morici G, Riccobono L, Insalaco G, Bonanno A, Profita M, Paterno A, Vassalle C, Mirabella A, and Vignola A. Airway inflammation in nonasthmatic amateur runners. *Am J Physiol Lung Cell Mol Physiol* 281: L668-676, 2001.
5. Bonsignore M, Morici G, Vignola A, Riccobono L, Bonanno A, Profita M, Abate P, Scichilone N, Amato G, Bellia V, and Bonsignore G. Increased airway inflammatory cells in endurance athletes: what do they mean? *Clinical Exp Allergy* 33:14-21, 2003.
6. Boushnel R, Henning L, Olesen J, Nowak M, Simonsen L, Bulow J, Ljaer M. Regional blood flow during exercise in humans measured by near-infrared spectroscopy and indocyanine green. *J Appl Physiol* 89: 1868-1878, 2000.
7. Bradley B, Azzawi M, Jacobson M, Assoufi B. Collins JV, Irani AM, Schwartz LB, Durham SR, Jeffery PK, and Kay AB. Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects

without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. *J.Allergy Clin Immunol* 88: 661-674, 1991.

8. Caillaud C, Serre-Cousine O, Anselme F, Capdevilla X, and Prefaut C. Computerized tomography and pulmonary diffusing capacity in highly trained athletes after performing a triathlon. *J Appl Physiol* 79: 1226-1232, 1995.
9. Chimenti L, Morici G, Paterno A, Bonanno A, Siena L, Licciardi A, Veca M, Guccione W, Macaluso F, Bonsignore G, and Bonsignore M. Endurance training damages small airway epithelium in mice. *Am J Respir Crit Care Med* 175: 442-449, 2007.
10. Chirpaz-Oddou MF, Favre-Juin A, and Flore P. Nitric Oxide response in exhaled air during an incremental exhaustive exercise. *J Appl Physiol* 82: 1311-1318, 1997.
11. Cianchetti S, Bacci E, Ruocco L, Bartoli L, Carnevali S, Dente FL, Di Franco A, Giannini D, Scuotri L, Vagaggini B, and Paggiaro PL. Salbutamol pretreatment does not change eosinophil percentage and eosinophilic cationic protein concentration in hypertonic saline-induced sputum in asthmatic subjects. *Clinical and Experimental Allergy* 29: 712-718, 1999.
12. Dahlen, SE. In: Leukotrienes. Inflammatory Mechanisms in Asthma, edited by Holgate ST and Busse WW. Pp. 679-720, 1998.
13. Davis MS, Malayer JR, Vandeventer L, Royer CM, McKenzie EC, Williamson KK. Cold weather exercise and airway cytokine expression. *J Appl Physiol* 98: 2132-2136, 3005.

14. Dempsey JA, Hanson PG, and Henderson KS. Exercise-induced arterial hypoxaemia in healthy human subjects at sea level. *J Physiol* 355: 161-175, 1984.

15. Dempsey JA, and Wagner PD. Exercise-induced arterial hypoxemia. *J Appl Physiol* 87: 1997-2006, 1999.

16. Edwards MR, Hunte GS, Belzberg AS, Sheel AW, Worsley DF, and McKenzie DC. Alveolar epithelial integrity in athletes with exercise-induced hypoxemia. *J Appl Physiol* 89: 1537-1542, 2000.

17. Eggleston PA, Rosenthal RR, Anderson SA, Anderton R, Bierman CW, Bleecker ER, Chai H, Cropp GJ, Johnson JD, Konig P, Morse J, Smith LJ, Summers RJ, and Trautlein JJ. Guidelines for the methodology of exercise challenge testing of asthmatics. *J Allergy Clin Immunol* 64: 642-645, 1979.

18. Eldridge MW, Braun RK, Yoneda KY, and Walby WF. Effects of altitude and exercise on pulmonary capillary integrity: evidence for subclinical high altitude pulmonary edema. *J Appl Physiol* 100: 972-980, 2006.

19. Eldridge MW, Braun RK, Yoneda KY, Walby WF, Bennett S, and Hyde DM. Lung injury after heavy exercise at altitude. *Chest* 114: 66S-67S, 1998.

20. Fahy JV, Liu J, Wong H, and Boushey HA. Cellular and biochemical analysis of induced sputum from asthmatic and from healthy subjects. *Am Rev Respir Dis* 147: 1126-1131, 1993.

21. Fahy JV, Wong H, Liu J, and Boushey HA. Comparison of samples collected by sputum induction and bronchoscopy from asthmatic and healthy subjects. *Am J Respir Crit Care Med* 152: 53-58, 1995.

22. Freed AN. Models and Mechanisms of exercise-induced asthma. *Eur Respir J* 8: 1770-1785, 1995.

23. Freed AN, Davis MS. Hyperventilation with dry air increases airway surface fluid osmolality in canine peripheral airways. *Am J Respir Crit Care Med* 159: 1101-1107, 1999.

24. Gershman NH, Liu H, Wong HH, Liu JT, and Fahy JV. Fractional analysis of sequential induced sputum samples during sputum induction: Evidence that different lung compartments are sampled at different time points. *J Allergy Clin Immunol* 104: 322-328, 1999.

25. Hanel B, Law I, and Mortensen J. Maximal rowing has an acute effect on the blood-gas barrier in elite athletes. *J Appl Physiol* 25: 25, 2003.

26. Helenius IJ, Rytila P, Metso T, Haahtela T, Venge P, and Tikkanen HO. Respiratory symptoms, bronchial responsiveness, and cellular characteristics of induced sputum in elite swimmers. *Allergy* 53: 346-352, 1998.

27. Holz O, Jorres RA, Koschyk S, Speckin P, Welker L, and Magnussen H. Changes in sputum composition during sputum induction in healthy and asthmatic subjects. *Clinical and Experimental Allergy* 28: 284-292, 1998.

28. Hopkins SR, Belzberg AS, Wiggs BR, and McKenzie DC. Pulmonary transit time and diffusion limitation during heavy exercise in athletes. *Respir Physiol* 103:67-73, 1996.

29. Hopkins SR, Gavin TP, Siafakas NM, Haseler LJ, Olfert IM, Wagner H, and Wagner PD. Effect of prolonged, heavy exercise on pulmonary gas exchange in athletes. *J Appl Physiol* 85:1523-1532, 1998.

30. Hopkins SR, McKenzie DC, Schoene RB, Glenny RW, and Robertson HT. Pulmonary gas exchange during exercise in athletes. I.Ventilation-perfusion mismatch and diffusion limitation. *J Appl Physiol* 77: 912-917, 1994.

31. Hopkins SR, Schoene RB, Henderson WR, Spragg RG, Martin TR, and West JB. Intense exercise impairs the integrity of the pulmonary blood-gas barrier in elite athletes. *Am J Respir Crit Care Med* 155: 1090-1094, 1997.

32. Hopkins SR, Schoene RB, Henderson WR, Spragg RG, and West JB. Sustained submaximal exercise does not alter the integrity of the lung blood-gas barrier in elite athletes. *J Appl Physiol* 84: 1185-1189, 1998.

33. Jatakanon A, Lim S, Chung KF, and Barnes PJ. An inhaled steroid improved markers of airway inflammation in patients with mild asthma. *Eur Respir J* 12: 1084-1088, 1998.

34. Karjalainen E, Laitinen A, Sue-Chu M, Altraja A, Bjermer L, and Laitinen L. Evidence of airway inflammation and remodeling in ski athletes with and without bronchial hyperresponsiveness to methacholine. *Am J Respir Crit Care Med* 161: 2086-2091, 2000.

35. Keatings VM, Evans DJ, O'Connor BJ, and Barnes PJ. Cellular profiles in asthmatic airways: a comparison of induced sputum, bronchial washings, and bronchoalveolar lavage fluid. *Thorax* 52: 372-274, 1997.

36. Kips JC, Inman MD, Jayaram L, Bel EH, Parameswaran K, Pizzichini MM, Pavord ID, Djukanovic R, Hargreave FE, and Sterk PJ. The use of induced sputum in clinical trials. *Eur Respir J* 20: 47s-50s, 2002.

37. Konig P. Exercise challenge: indications and techniques. *Allergy Proc* 10(5): 345-348, 1989.

38. Larsson K, Ohlsen L, Larsson P, Malmberg P, Rydstrom P, and Ulriksen H. High prevalence of asthma in cross country skiers. *B.M.J.* 307: 1326-1329, 1993.

39. Lacy P, Lee JL, Vathanayagam D. Sputum analysis in diagnosis and management of obstructive airway diseases. *Ther Clin Risk Manag*. Sept; 1(3)169-79, 2005.

40. Levine BD, Lane LD, Buckey JC, Friedman DB, and Blomqvist CG. Left ventricular pressure-volume and frank-starling relations in endurance athletes. Implications for orthostatic tolerance and exercise performance. *Circulation* 84: 1016-1023, 1991.

41. Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, and Djukanovic R. The relationship between airways inflammation and asthma severity. *Am J Respir Crit Care Med* 161: 9-16, 2000.

42. Maestrelli P, Saetta M, Stefano AD, Calcagni PG, Turato G, Ruggieri MP, Roggeri A, Mapp CE, and Fabbri LM. Comparison of leukocyte counts in sputum, bronchial biopsies, and bronchoalveolar lavage. *Am J Respir Crit Care Med* 152: 1926-1931, 1995.

43. Manier G, duclos M, Arsac L, Moinard J, andLaurent F. Distribution of lung density after strenuous, prolonged exercise. *J Appl Physiol* 87: 83-89, 1999.

44. Maroun MJ, Mehta S, Turcotte R, Cosio MG, Hussain SN. Effects of physical conditioning on endogenous nitric oxide output during exercise. *J Appl Physiol* 79: 1219-1225, 1995.

45. McFadden ER. Hypothesis: exercise-induced asthma as a vascular phenomenon. *Lancet* 335: 880-883, 1990.

46. McKechnie JK, Leary WP, Noakes TD, Kallmeyer JC, MacSearraigh ET, and Olivier LR. Acute pulmonary oedema in two athletes during a 90-km running race. *S Afr Med J* 56: 261-265, 1979.

47. McKenzie DC, Lama IL, Potts JE, Sheel AW, and Coutts KD. The effect of repeat exercise on pulmonary diffusing capacity and EIH in trained athletes. *Med Sci Sports Exerc* 31: 99-104, 1999.

48. Morici G, Bonsignore M, Zangla D, Loredana Riccobono L, Profita M, Bonanno A, Paterno A, Giorgi R, Mirabella F, Chimenti L, Benigno A, Vignola A, Bellia V, Amato G, and Bonsignore G. Airway cell composition at rest and after an all-out test in competitive rowers. *Med Sci Sports Exercise* 36: 1723-1729, 2004.

49. Mulvey DA, Koulouris NG, Elliott MW, Laroche CM, Moxham J, and Green M. Inspiratory muscle relaxation rate after voluntary maximal isocapnic ventilation in humans. *J Appl Physiol* 70(5): 2173-2180, 1991.

50. Pavord ID, Sterk PJ, Hargreave FE, Kips JC, Inman MD, Louis R, Pizzichini MM, Bel EH, Pin I, Grootendorst DC, Parameswaran K, and Djukanovic R. *Eur Respir J* 20: 40s-43s, 2002.

51. Paggiaro PL, Chanez P, Holz O, Ind PW, Djukanovic R, Maestrelli P, Sterk PJ. Sputum Induction. *Eur Respir J* 20: Suppl 37 3s-8s, 2002.

52. Pin I, Gibson PG, Kolendowicz R et al. Use of induced sputum cell counts to investigate airway inflammation in asthma. *Thorax* 47: 25-29, 1992.

53. Pizzichini E, Pizzichini MM, Efthimiadia A, Evans S, Morris MM, Squillace D, Gleich GJ, Dolovich J, and Hargreave FE. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. *Am J Respir Crit Care Med* 154: 308-317, 1996.

54. Pizzichini E, Pizzichini MM, Leigh E, Djukanovic R, and Sterk PJ. Safety of sputum induction. *Eur Respir J* 20: Suppl 37, 9s-18s, 2002.

55. Power C, Sreenan S, Hurson B, Burke C, and Poulter L. Distribution of immunocompetent cells in the bronchial wall of clinically healthy subjects showing bronchial hyperresponsiveness. *Thorax* 48: 1125-1129, 1993.

56. Prefault C, Anselme F, Caillaud C, and Masse-Biron J. Exercise-induced hypoxemia in older athletes. *J Appl Physiol* 76: 120-126, 1994.

57. Reeves JT, Groves BM, Cymerman A, Sutton JR, Wagner PD, Turkevich D, and Houston CS. Operation Everest II: cardiac filling pressures during cycle exercise at sea level. *Respir Physiol* 80: 147-154, 1990.

58. Reeves JT and Taylor AE. Pulmonary hemodynamics and fluid exchange in lungs during exercise. In: *Handbook of Physiology. Exercise: Regulation and Integration of Multiple Systems.* Bethesda, MD: Am. Physiol Soc., 1996, sect 12, chapt. 17, p.770-838.

59. Rundell KW, Wilber RL, Szmedra L, Jenkinson DM, Mayers LB, and Im J. *Med Sci Sports Exerc* 32: 309-316, 2000. Exercise-induced asthma screening of elite athletes: field versus laboratory exercise challenge.

60. Spooner C, Rowe BH, Saunders LD. Nedocromil sodium in the treatment of exercise-induced asthma: a meta-analysis. *Eur Respir J* 16: 30-37, 2000.

61. Stickland MK and Lovering AT. Exercise-induced intrapulmonary arteriovenous shunting and pulmonary gas exchange. *Exerc. Sport Sci Rev* 34: 99-106, 2006.

62. Stickland MK, Welsh RC, Haykowsky MJ, Petersen SR, Anderson WD, Taylor DA, Bouffard M and Jones RL. Intra-pulmonary shunt and pulmonary gas exchange during exercise in humans. *J Physiol* 561: 321-329, 2004.

63. Stickland MK, Welsh RC, Haykowsky MJ, Petersen SR, Anderson WD, Taylor DA, Bouffard M and Jones RL. Effect of acute increases in pulmonary vascular pressures on exercise pulmonary gas exchange. *J Appl Physiol* 100: 1910-1917, 2006a.

64. Stickland MK, Welsh RC, Petersen SR, Tyberg JV, Anderson WD, Jones RL, Taylor DA, and Haykowsky MJ. Does fitness level modulate the cardiovascular hemodynaminc response to exercise? *J Appl Physiol* 100: 1895-1901, 2006b.

65. Sue-Chu M, Larsson L, Moen T, Rennard S, Bjermer L. Bronchoscopy and bronchoalveolar lavage findings in cross-country skiers with and without "ski asthma." *Eur Respir J* 13: 626-632, 1999.

66. Wagner PD, Gale GE, Moon RE, MmHge-Bueno JR, Stolp BW, and Saltzman HA. Pulmonary gas exchange in humans exercising at sea level and simulated altitude. *J Appl Physiol* 61: 260-270, 1986.

67. West JB. Invited Review: pulmonary capillary stress failure. *J Appl Physiol* 89: 2483-2489; discussion 2397, 2000.

68. West JB. Vulnerability of pulmonary capillaries during exercise. *Exerc Sport Sci Rev* 32: 24-30, 2004.

69. West JB, and Mathieu-Costello O. Stress failure of pulmonary capillaries: role in lung and heart disease. *Lancet* 340: 762-767, 1992.

70. West JB, and Wagner PD. Pulmonary gas exchange. *Am J Respir Crit Care Med* 157: S82-87, 1998.

71. Wetter TJ, St Croix CM, Pegelow DF, Sonetti DA, and Dempsey JA. Effects of exhaustive endurance exercise on pulmonary gas exchange and airway function in women. *J Appl Physiol* 91: 847-858, 2001.

72. Wetter TJ, Xiang Z, Sonetti DA, Haverkamp HC, Rice AJ, Abbasi AA, Meyer KC, Dempsey JA. Role of lung inflammatory mediators as a cause of exercise-induced arterial hypoxemia in young athletes. *J Appl Physiol* 93: 116-126, 2002.

73. Young M, Sciurba F, and Rinaldo J. Delirium and pulmonary edema after completing a marathon. *Am Rev Respir Dis* 136: 737-739, 1987.

Chapter 3

Pulmonary Damage and Airway Inflammation in Trained and Untrained Subjects Following an Acute Maximal Exercise Bout

3.1 Introduction

The pulmonary blood-gas barrier must be thin enough to decrease the resistance to gas exchange and robust enough to withstand increased capillary wall pressures which develop when capillary pressure increases with exercise (West, 2000; Wagner, 1986). However, the resulting increase in pulmonary capillary pressure with exercise may be excessive and lead to exercise-induced pulmonary damage (West, 2000). As pulmonary artery pressure rises, fluid moves from the capillary lumen into the alveolar wall interstitium and possibly into the alveolar space (West, 2004). This induced pulmonary edema would become more severe as PAP increases as a result of high capillary transmural pressures pulmonary rupture or damage to the capillary endothelial and alveolar epithelial cell layers may occur (West, 2004). Hopkins et al. (1997) has shown increased concentrations of red blood cells and proteins in bronchoalveolar lavage fluid of highly trained athletes following high intensity exercise that was evidence of alveolar damage that could alter the blood gas barrier and limit diffusion capacity.

Other research using a direct measurement of pulmonary vascular pressures during exercise has shown that pulmonary venous pressure was lower in endurance trained athletes during exercise as compared to sedentary subjects (Stickland, 2006). Levine et al. (1991) demonstrated that at the same pulmonary artery wedge pressure, athletes had a higher stroke volume (SV) than controls using lower body negative pressure and rapid saline infusion in subjects while at rest. Thus, while some human evidence of ruptured pulmonary capillaries during exercise exists, the nature and extent of this damage is still

disputed (Edwards et al, 2000; Eldridge et al, 2006; Hanel et al, 2003; Hopkins et al, 1997; Hopkins et al, 2007) and it remains to be determined whether less fit subjects experience greater exercise-induced lung damage as compared to endurance trained subjects when performing maximal exercise.

In addition to pulmonary damage, it has been speculated that airway inflammation can occur as a result of high ventilation during exercise could produce airway edema, excessive mucus production, and smooth muscle constriction in the small airways, resulting in a reduced distribution of alveolar ventilation during exercise (Morici et al, 2004; Wetter et al, 2001). Increased neutrophils, eosinophils, and lymphocytes alone or in combination were reported in the airways of runners (Bonsignore, 2001), swimmers (Helenius, 1998), and skiers (Karjalainen, 2000). Bonsignore et al. (2001) found high polymorphonuclear neutrophil counts in induced sputum of five middle aged, amateur runners following a marathon. In addition, Morici et al (2004) collected induced sputum samples in a group of young competitive rowers at rest and within 1 hour after a short all-out rowing test over 1000m (about 3min) and found that post-exercise total corrected cell counts were highest in athletes showing the highest minute ventilation per kilogram and tidal volume per kilogram during exercise. These findings supported the possibility that high airflows during all-out exercise may cause epithelial damage and inflammation through shear stress on the airway wall (Chimenti et al, 2007; Morici et al, 2004). Most studies however, obtained airway cell samples in athletes only at rest. Recently increased or unchanged neutrophil counts compared with baseline have been documented in non-asthmatic endurance athletes after competitions (Bonsignore 2001). Unfortunately,

limited data are available in human subjects on the relationship between exercise ventilation and post-exercise airway cell counts or composition.

The purpose of this study was to examine the composition of airway and lung cells using simple sputum induction at rest and following acute maximal exercise in endurance trained (ET) and non-aerobically trained (UT) male participants. It was hypothesized that endurance trained subjects would have decreased presence of red blood cells (indicating lung damage) and increased presence of inflammatory cell markers (neutrophils and bronchial epithelial cells) in their sputum sample following maximal exercise. In addition, at the same average absolute power output, it was hypothesized that endurance trained men would have fewer red blood cells and fewer markers of airway inflammation (as above) present in their sputum than untrained men.

3.2 Methods

Subjects and Experimental Design

Fifteen healthy, non-asthmatic, men that were not participating in regular aerobic endurance exercise [UT mean age (± SD)= 21.7 (3.3) yrs, height= 177(9.0)cm, weight=80.8 (16.5)kg, $\dot{V}O_2$ peak = 43.0 (3.7) $ml \times kg^{-1} \times min^{-1}$] and 11 healthy, non-asthmatic, endurance trained athletes [ET mean age (± SD)= 25.4 (4.5) yrs, height=180 (6.6)cm, weight= 71.7 (7.2)kg, $\dot{V}O_2$ peak = 62.6 (5.9) $ml \times kg^{-1} \times min^{-1}$] provided written informed consent that was approved by a Health and Research Ethics Board and a physical activity readiness questionnaire (PAR-Q). Subjects were of various ethnic origins and backgrounds in both groups. All untrained subjects were not regular exercisers with minimal aerobic endurance experience [as evaluated by the Godin Leisure-Time Exercise Questionnaire (GLTEQ), Godin & Shephard, 1985]. All

endurance trained athletes exercised strenuously a minimum of four days per week and trained as cyclists, runners, and/or triathletes (GLTEQ).

UT subjects performed 4 testing sessions, while ET subjects performed 5 testing sessions. Sessions were as follows: (1) Screening questionnaire, graded exercise test and basic spirometry to screen for resting lung function abnormalities as well as exercise-induced bronchospasm; (2) simple sputum induction at rest; (3) a 5km all-out cycling time trial followed by simple sputum induction two hours after exercise; and, (4) full pulmonary function testing using plethysmography (lung volumes and diffusion capacity) at the University of Alberta Hospital. ET subjects performed the same four sessions as the UT group, and in addition, these subjects performed an additional 5km cycling time trial at the same exercise intensity (average absolute power output over three stages of time trial) as the UT subjects did during their 5km time trial with simple sputum induction 2 hours following exercise. For the ET group, the order of the two time trials was randomized. Induced sputum measurements either at rest or following exercise were, at minimum, 7 days apart. In addition, each subject was scheduled at the same time of day for each testing session.

A verbal explanation of all procedures was also done prior to commencing the test protocol procedures. Subjects were screened for any medical conditions or contraindications such as asthma, allergies, or a history of smoking using a questionnaire in addition to baseline spirometry. All subjects had no symptoms of upper respiratory tract infections or cold/flu like symptoms within the four weeks preceding the experimental tesithg. Forced vital capacity (FVC), one-second forced expiratory volume (FEV_1) and forced expiratory flow between 25 and 75% of FVC (FEF 25-75%) were

obtained using Office Medic Spirocard system (QRS Diagnostics, Plymouth, MN). Airway obstruction was defined as an FEV1/FVC ratio of less than 80% predicted.

Maximal Exercise Test and EIB screening

Each subject arrived at the lab for his first exercise test in the rested state. All subjects were instructed to eat/drink ad libitum 2 to 3 hours before their exercise test. Height was measured using a wooden plane placed on the head with the subjects standing upright against a wall and body mass was measured using a balance beam scale. Age and race were recorded. Subject's were "fitted" on the cycle ergometer by adjusting seat height and handle bars. Before starting the exercise warm-up, subjects were given a brief explanation of the exercise protocol. The warm-up for the untrained subjects was 100W and 75rpm for 5min and 140W and 75 rpm for 5min for the trained subjects. The graded exercise test (GXT) consisted of incremental exercise to volitional exhaustion on a Velotron Racer Mate (Seattle, Washington). Pedal rate was kept constant at 75rpm, power output was increased 40W every 2 minutes until ventilatory threshold was reached by a visual determination of a systematic increase in both V_E/V_{O2} and V_E/VC_{O2} ratios (during a real time graphical display on the metabolic measurement system computer) after which power output was increased 40W every minute until exhaustion. During the test, each subject wore a headgear apparatus and a one-way mouthpiece (Hans-Rudolph valve, Kansas) to collect expired air which was analyzed in a metabolic measurement system (ParvoMed True Max 2400, Utah) that was calibrated for gas concentration (O_2 and CO_2) and volume of air according to the manufacturer. Volitional exhaustion was defined as the point at which the subject could not continue to exercise due to fatigue despite further extrinsic motivation. Peak oxygen consumption ($\dot{V}O_2$ peak) was the

highest amount of oxygen consumed per minute during the graded exercise test (20sec averaging), that was associated with a respiratory exchange ratio great than 1.1 and near attainment of age-predicted or attainment of known maximum heart rate.

Heart rate, (Polar Electro, Kempele, Finland), pedal speed, and power output were recorded every minute. Spirometry was performed at rest before the test and at 1min, 3min, 5min, 10min, and 15min after exercise to assess exercise-induced bronchospasm (EIB) which is defined as a decrease in forced expiratory volume of air in 1 second (FEV_1) of >10% from the baseline value (ATS, 1995). Subjects who experienced EIB, who had abnormal resting spirometry or medical contraindications such as allergies were excluded from this study. Two subjects were excluded from the study for having abnormal baseline spirometry.

Simple Sputum Induction and Processing

Simple sputum induction and processing were according to a modified version of Fahy et al.(1993) as reported by Bonsignore et al (2001). Prior to sputum induction, baseline resting spirometry was repeated three times as well as 15 minutes following the inhalation of Ventolin®, a bronchodilator, using 2 doses separated by 30seconds from a metered-dose inhaler device. The subject was then instructed to blow their nose and rinse their mouth with water. The subject was instructed to sit next to a Biological safety cabinet (FORMA Class II, A2). The ultrasonic nebulizer was filled with 9mL of 3% hypertonic saline and the subject was instructed to inhale the aerosol through the mouthpiece, breathing deeply and slowly. Subjects were told that it was acceptable to cough whenever necessary. After 7 minutes, the nebulizer was stopped and subjects blew their nose and rinsed their mouth with water. They were then instructed to breathe deeply

and cough phlegm into a sterile sample container trying to acquire at least 2mL of sputum. Subjects were instructed to spit any saliva into the sink so as to avoid squamous cell contamination of their sample. After this, 3 forced vital capacities were performed and if FEV_1 decreased by no more than 10% (which is expected after this procedure) another sputum induction with 9cc of 4% was performed for 7min, followed by a third with 5% hypertonic saline for 7min provided no change in FEV_1 was observed. There were no subjects who had a drop in their FEV_1 during the sputum induction.

Sputum processing was completed in the pulmonary Research Lab that was set-up to process such samples. Selected sputum plugs were weighed and mixed with 4x (weight: volume) with a 1:10 dilution of Dithiothreitol (DTT) with distilled water. The sample was vortexed for 10-15 seconds and placed on a rocker for exactly 15 min. Samples weighing more than 250mg were mixed several times during the rocking process. After 15 minutes, the specimen was vortexed for 10-15 seconds and Dulbecco's Phosphate Buffered Saline (DPBS), at 4x dilution factor (original weight:volume) was added to the sample and vortexed again for 10-15 seconds. The sample was then filtered (4cm x 4cm square of 48-53 micron nylon mesh) and cell viability was assessed with a 10µL sputum solution sample and 10µL Trypan blue in a small microfuge tube. This mixture was added to a haemocytometer and the cell concentration, % squamous cells, absolute cell count, absolute cell count per mg sputum, non-squamous cell viability, and total cell counts were calculated. The remaining sputum was spun in a centrifuge (Beckman TJ-6) for 5 min at 450 rpm. 500 µL of supernatant was then transferred into two microfuge tubes and stored -70°C. DPBS was then added to the remaining fresh sample to create a cell concentration of 0.7×10^6 cells per mL. This sample was vortexed

for 10-15 seconds and 50 μL of sample was withdrawn and centrifuged for 5 minutes at 450rpm (Shandon Cytospin 3 Centrifuge).

Slides were stained with Diff Quick (Dade Behring AG, Switzerland) by the same trained technician who then counted the first 400cells/slide. The technician was blinded to the sample, subjects, and experimental protocol. Sputum samples were considered adequate if the sputum volume was at least 1ml with <50% squamous cells on differential count. Cell density was calculated by dividing the number of non squamous cells by the quadrants counted (2-9) on the haemocytometer x the amount the each quadrant held (0.1×10^{-6}). Cell density was then multiplied by the weight of the filtered sample in grams and converted to million cells/mL. Figure 3.1 shows a representative stain of an ET subject at rest and following both of his time trials. Figure 3.2 is an example of red blood cells in sputum. Differential cell counts (including red blood cells) were assessed by one trained technician for the first 400 cells.

Three randomly selected slides were counted following the completion of data collection and analysis of this study. The sputum cell counts were reliable and repeatable as indicated by the coefficient of variation (Appendix F).

Experimental exercise protocol: 5km Time Trial

The testing protocol was designed to be similar to that previously shown to cause exercise-induced lung damage (Hopkins et al., 1997). Each subject adhered to the same pretest guidelines as previously mentioned for the graded exercise test. Subjects came in for the time trial at the same time as for their baseline non-exercise control sputum measurements. The seat height and handle-bar position were adjusted to that of their previous test or personal preference for the ET subjects. The ET participants used their

own clip-less pedals and cycling shoes. All subjects performed a standardized 10min warm-up: 5 min at 30% and 5 min at 40% of their maximal power output attained during their $\dot{V}O_2$ peak test. After the warm-up, subjects were given two minutes to stretch and were then fitted with a Polar HR monitor and forehead pulse oximeter probe (Nellcor N-595, CT). Prior to starting the exercise protocol, subjects were seated on the bike at rest for at least 1minute to ensure all equipment and technical measurements were operational. All subjects started from rest and performed a 5km cycling time trial (TT) as fast as they could. The time trial included an initial 0.5km section of 0% grade followed by a 12% grade for 1km. The last 3.5km was at 0% grade. Strong verbal encouragement was constantly provided to each subject. The temperature and humidity averaged 22˚C and 20% respectively for all testing. An electric fan was directed at the subject for comfort and they were not allowed to drink water during the time trial. Power output, heart rate, hemoglobin saturation (SpO_2), cadence, respiratory rate, minute ventilation, and oxygen consumption (as previously described) were recorded continuously and averaged every 10 seconds. After completion of the time trial, final time, average power output, average cadence, and average speed (kph) were recorded. For the time interval between the end of the TT and the 2 hour post-exercise sputum test, subjects were allowed to drink water as libitum but no other type of beverage or food of any kind was allowed. As well, absolutely no exercise was done within this time and subjects were instructed to minimize the time they spent outdoors. Conditions for obtaining the baseline non-exercise control sputum sample and all of the sputum samples following exercise remained the same.

Trained subjects performed an additional 5km cycling exercise test at the same average absolute intensity as the untrained subjects' 5km all-out time trial on a different day, at least one week following their last exercise test. For this "moderate" intensity time trial the trained subjects were given the mean power output of the untrained group over three sections of the time trial (flat 0.5km, hill 1km, flat 3.5km) and were instructed to maintain as close to that mean power output for each of the three sections. The order of testing for the two 5 km time trials performed by the trained subjects was randomized and therefore, testing of the ET group could only begin following the full completion of the UT group. The protocol for this 5km time trial was executed exactly the same as for the all-out time trial, the only difference being that the power output was controlled and predetermined. Simple sputum induction was performed again according to the previously mentioned protocol.

Pulmonary Function Testing

Ten untrained and eight endurance trained subjects had full pulmonary function testing done at the University of Alberta Hospital Pulmonary Function Laboratory on a separate day under the supervision of qualified respiratory theraptists. Lung function testing was not done on entire subject population due to lack of compliance. Lung function testing included spirometry, lung volumes (TLC using plethysmography), and diffusion capacity (DLCO and DLCO adjusted for V_A).

Statistical Analysis

Means and standard deviations were calculated for all metabolic measures, time trial values, and sputum differential cell counts. Medians and inter-quartile ranges were calculated on absolute sputum cell counts. A Students' independent t-test was used to

determine any significant difference between the two groups on the demographic and fitness variables. One-way analysis of variance (ANOVA) was used to determine whether there were any significant differences between the ET and UT groups for the dependent variables associated with the time trial performance test. One-way ANOVA with repeated measures was used to compare the two different time trial performance test variables for the ET group.

Separate two-way ANOVA with repeated measures were used to determine if there were any significant differences in the sputum cell counts between groups at rest and following exercise. Due to the nature of the experimental design, three separate comparisons were necessary. First, a 2 group (UT vs. ET) x 2 time (rest, post-exercise) ANOVA with repeated measures on one factor (time) was used to compare rest and post-exercise variables between the UT and the ET groups during the time trial test (ET-HI). The second comparison of rest and post-exercise variables was made between the UT and the ET group matched at the same absolute time trial intensity averaged by the UT group (ET-MOD). Finally, the third compared the sputum cell counts at rest and after exercise in both conditions performed by the ET group (ET-HI versus ET-MOD). Any significant F-ratios revealed by ANOVA were further analyzed with a Newman-Keuls multiple comparison procedure. Pearson's correlation coefficient was calculated between all variables. For all statistics, an alpha of $p<0.05$ was considered significant, a priori.

3.3 Results

Participant Characteristics

There were no significant differences in mean height and body mass between the UT and ET groups (Table 3.1). Participants in the ET group were 4 years older than in

the UT group and exercised strenuously a minimum of four days per week as was indicated by their score on the GLTEQ ($P<0.05$). Mean absolute and relative $\dot{V}O_2$ peak of the ET group was significantly higher than the UT group. There were no significant differences in FVC and FEV_1, TLC, DLCO, and DLCO adjusted for V_A between groups. One UT subject was excluded from the study due to lower airway obstruction that was indicated on his pulmonary function test.

Time Trial Performance Measurements

There were significant differences in ventilatory, metabolic, and performance measures during the all-out time trials between groups (Table 3.2). Trained subjects performed at a significantly higher relative and absolute $\dot{V}O_2$ and had significantly higher relative and absolute minute ventilation, power output, and speed in comparison to the untrained group. The ET group had significantly lower oxygen saturation and tidal volume compared to the UT group.

There were no significant differences in power output and absolute $\dot{V}O_2$ between the UT all-out time trial and the ET moderate time trial. ET athletes had significantly higher relative $\dot{V}O_2$ and lower V_E, RR, HR, and performed the TT at a lower percentage of their $\dot{V}O_2$ peak ($P<0.05$). There were expected significant differences between the two time trials of the ET group with regard to all performance measures (Table 3.2).

Differential Sputum Cell Counts

In the UT group, 4 subjects were unable to produce sputum at rest. All of the subjects were able to produce viable sputum samples following their time trials. In the ET group, one subject was unable to produce satisfactory sputum at rest and following his moderate intensity time trial and another subject was unable to produce a satisfactory

sputum sample following his moderate intensity time trial. All ET subjects were able to produce sputum following the all-out time trial.

Table 3.3 and 3.4 display the sputum cell counts for all cells at rest and these were considered to normal for the UT group (Belda et al, 2000). There was no statistical difference in eosinophil, neutrophil, and red blood cell percentages between rest and post-exercise sputum cell counts in the UT group (Table 3.3). Lymphocytes, squamous cells and alveolar macrophages were lower and bronchial epithelial cells were higher following the all-out time trial in UT participants ($P<0.05$). Total cell count was increased following the all-out time trial as compared to rest ($P<0.05$) in the UT group. No UT subjects produced red blood cells in their sputum at rest and four subjects produced red blood cells in their sputum following their time trial.

Sputum differential cell counts for the ET group were also considered to be within a normal range at rest (Belda et al, 2000). There were no significant differences in eosinophils and neutrophils and red blood cells between rest and either exercise counts (rest vs. TT-MOD and/or TT-HI) or between both time trial counts (TT-MOD vs. TT-HI). The ET group had significantly higher neutrophil differential cell count than the UT group at rest. Lymphocytes significantly decreased following the TT-MOD as compared to rest and further significantly decreased from both rest and TT-MOD following the TT-HI. In addition, the lymphocyte cell count following the ET TT-MOD was significantly higher than the lymphocyte cell count following the UT TT-HI (Table 3.3). Alveolar macrophages and squamous cells were significantly lower after the TT-HI compared to both the baseline non-exercise control and TT-MOD conditions. The alveolar macrophages following TT-MOD were significantly different from both the UT TT-HI

and ET- TT-HI. In addition, bronchial epithelial cells following TT-MOD and TT-HI in the ET group were significantly higher than the baseline non-exercise control cell count and significantly different from each other. Total cell count was significantly higher than the baseline non-exercise control total cell count in the ET group. At rest, one ET subject had a single red blood cell in his sputum sample. Four ET subjects produced red blood cells in their sputum following their moderate time trials (TT-MOD) and three produced red blood cells following their maximal time trials (TT-HI).

Bronchial epithelial cells were different at rest and following both time trials in both groups ($P<0.05$). There was a significant decrease in lymphocytes and alveolar macrophages and a significant increase in bronchial epithelial cells when comparing (UT and ET) rest vs. ET TT-MOD and when comparing (UT and ET) rest vs. (UT and ET) TT-HI. Total cell count was not significantly different between groups but was increased from rest in both groups following TT-HI.

Correlations

There was no correlation with baseline non-exercise control sputum cell count data and time trial metabolic and ventilatory measures. There was a significant correlation with the ET relative minute ventilation and bronchial epithelial cells following TT-MOD and TT-HI combined ($r= 0.47$, $P<0.05$; Figure 3.4). In addition, there was also a significant correlation ($r= 0.39$, $p<0.05$) in neutrophil cell counts post-exercise in the ET and the UT group combined and V_E /kg (Figure 3.4). There was a negative correlation with both absolute and relative all-out time trial V_E (L and $L \times kg^{-1}$) and lymphocytes and alveolar macrophages ($r = -0.57$ and $r = -0.65$ and $r=-0.59$ and $r=-0.66$ respectively; $p<0.05$). In addition, a negative correlation between mean time trial HR and

lymphocytes and macrophages was observed (r =-0.75 and r = -0.68, respectively; P<0.05).There was a positive correlation between lymphocytes and FEV_1/FVC ratio (r= 0.60; P<0.05). (see Appendix D)

3.4 Discussion

This study was designed to assess whether acute exercise affects airway and pulmonary cell composition, and the possible relationship between fitness level and airway/lung differential cell counts. It has been hypothesized that exercise increases capillary pressure, alters the integrity of the blood gas barrier and increases permeability to red blood cells (Hopkins, 1997). Highly trained endurance athletes have reported hemoptysis and tasting of blood after exercise with high intensity, "race-type" efforts (MacKechnie et al, 1979). Exercise-induced pulmonary hemorrhage has been described in a top class rugby player where bronchoscopy revealed bleeding from the lung periphery (West et al, 1991). To date, a significant change in the presence of red blood cells in sputum samples of healthy subjects following exercise has not been reported. Although there was a numerical increase in red blood cells 2 hours post-exercise, this increase in both groups did not reach statistical significance. The proposed mechanism for the disruption of the pulmonary alveoli is that fluid overload and central vascular pooling combined with exercise, may raise pulmonary capillary pressure to the point where stress failure of the capillaries occurred. The lack of statistical significance in the present study's findings suggests that this did not occur in the majority of the trained or untrained subjects.

Induced sputum of endurance trained athletes at rest was rich in neutrophils as compared to untrained subjects but did not significantly change after exercise. There was

an increase in bronchial epithelial cells 2 hours following maximal intensity exercise in untrained and trained subjects. This supports our hypothesis that maximal exercise changes the composition of the cells lining the airways. Furthermore, as was hypothesized, elite athletes that exercised at the same average absolute power output during the time trial as untrained subjects' maximal intensity time trial produced fewer bronchial epithelial cells in their sputum 2 hours post-exercise. These findings may suggest a training adaptation in the airways of endurance trained athletes or it could be the result of a lower "relative" intensity of exercise and decreased minute ventilation required by the endurance trained athletes to perform the time trial at the same intensity as the untrained group.

The present study found that ET participants had a 22% higher number of neutrophils than UT subjects at rest. This is congruent with previous research that found airway neutrophil differential counts were increased in marathon runners (Bonsignore et al, 2001), swimmers (Bonsignore et al, 2003) and rowers (Morici et al, 2004) compared with sedentary controls in sputum cell counts at rest. Increased airway neutrophils were also found in bronchial biopsies of skiers at rest (Karjalainen, 2001). It appears that endurance training may play a direct role in the pathogenesis of airway neutrophilia in athletes. Because repeated dry air challenges in dog airways were associated with progressively increasing neutrophil recruitment (Freed and Davis, 2001), it has been speculated that repeated bouts of exercise may cause similar changes in humans undergoing regular training (Bonsignore et al, 2003). Bonsignore et al. suggest a state of "frustrated" airway inflammation in athletes because other than neutrophil influx, airway inflammatory cells of marathon runners and swimmers (Bonsignore et al, 2001, 2003)

were not activated at rest or after exercise. Long term consequences of participation in elite sports are unknown, but available evidence supports the hypothesis that endurance exercise may cause adaptive responses rather than airway damage. In this study, all of the participants were free from EIB so while the potential exists for airway inflammation to be present, this inflammation is not limiting to their training. Endurance trained athletes regularly expose themselves to greater levels of minute ventilation, which could potentially overwhelm the air-conditioning capacity of the upper airways.

An important finding of this study was that regardless of fitness level, both groups had significantly elevated total cell count and bronchial epithelial cells following their all-out time trial compared to rest. Furthermore, following the time trial that was matched to the untrained participants, the endurance trained athletes had bronchial epithelial cell counts that were higher than rest but lower than these counts 2 hours after the all-out time trial. This possibly indicates a "dose-response" of airway deconstruction and remodeling through the bronchial epithelial tissue. Because bronchial epithelial cell counts are still higher than at rest in the ET participants, the change in differential cell counts could merely indicate the athletes were working at a lower intensity and decreased V_E. Bronchial epithelial cell counts were similar between groups at both of their maximal intensities despite differences in respiratory rate, minute ventilation, and oxygen consumption. In addition, at the maximal power output of the UT group, the ET group has significantly lower percent of bronchial epithelial cells. As such, it remains possible that a training adaptation has occurred in ET athletes. While, Morici et al. (2004) did not reach statistical significance with changes in bronchial epithelial cells post-exercise, a

trend towards increased bronchial epithelial cells was identified and it was suggested with a larger sample size, the change would have reached statistical significance.

As for the mechanism(s) of exercise-induced airway changes in endurance athletes and untrained subjects following exercise, it appears that this may be partly linked to hyperventilation. The change in BECs showed a positive correlation with relative minute ventilation for the all-out time trial and moderate intensity time trial of the ET group. In addition, unlike the study by Morici et al, the present study also found a positive correlation in neutrophil cell counts after exercise and $VE \times kg^{-1}$ in both the ET and UT group. While this study was not designed to address the pathogenic mechanism(s) responsible for exercise-induced airway changes (and did not measure serum or sputum immunological markers) the positive correlation between bronchial epithelial cells, neutrophils and exercise minute ventilation suggests a possible mechanism for exercise- associated airway remodeling. The high airflows generated during all-out exercise may cause epithelial damage through shear stress on the airway wall. Exercise hyperventilation has been reported to be responsible for the cooling/re-warming process, epithelial cell dehydration (especially if exercise is performed in cold and/or dry air), and subsequent hyperosmolar stress (Bermon, 2007). Airway epithelial cells participate in local cytokine networks by synthesizing interleukins, chemokines, colony stimulating factors and growth factors in response to inflammatory mediators (Levine, 1995). Bronchial epithelial cell derived cytokines may thereby amplify ongoing inflammatory processes via the recruitment and activation of specific subsets of inflammatory cells, as well as by prolonging their survival in the airway microenvironment (Velden, 1998). However, exercise bronchodilation has been shown to

lower airway resistance and shear stress considerably and Crimi et al. (2002) argues against shear stress as a major role in airway epithelial damage. Further investigation into the hyperosmolarity of airway surface lining or cooling/re-warming of the bronchial wall during intense exercise is required to establish the role of hyperventilation in airway inflammation.

The results of this study also found that, besides bronchial epithelial cells and total cell count, the airway neutrophil was the cell type most affected by intense, short-lived exercise regardless of fitness level. Due to the increase in bronchial epithelial cells, both alveolar macrophages and lymphocytes decreased significantly in the sputum differential cell counts. There was no significant decrease in neutrophil differential cell counts following the all-out time trial in spite of the significant increase in bronchial epithelial cells. As such, it is likely if only pulmonary white blood cells were counted that a significant increase in neutrophils would have been evident. Bonsignore et al. (2001) reported an increase in neutrophils in marathon runners after exercise with changes only in white blood cells.

As well, the subjects in the present study were negative for EIB and yet still provided evidence for airway remodeling following exercise as demonstrated by the significant change in bronchial epithelial cells in both groups. Furthermore, ET athletes demonstrated significantly lower oxygen saturation during the all-out time trial than the UT group, with some athletes developing exercise-induced hypoxemia (ET range S_pO_2 = 89.9-89%) (Dempsey and Wagner, 1999). However, none of the mentioned findings were correlated with exercise-related respiratory symptoms. As such, increased numbers of inflammatory cells in the airways of ET and UT groups were not necessarily associated

with major clinical or functional alterations. To understand the physiological meaning of exercise-related airway changes in athletes and untrained subjects, further research is recommended.

The ventilatory and metabolic time trial data collected for both the UT and ET group reflected the significant difference between the groups with respect to fitness level and training experience. Based on the mean percentage of $\dot{V}O_2$ peak maintained by both participant groups for the all-out time trial, it was clear that the intensity of the time trial was relatively high for both groups. Despite having much a lower oxygen consumption, minute ventilation, and power output during their time trial, the UT group had a similar average heart rate and respiratory rate to the ET group. However, the differences in all other respiratory and metabolic variables between the UT and ET group are reflective of the high fitness of the ET group. The response of the ET group to the time trial at the same average power output as the UT group also attests to many physiological differences of the ET group as a result of their training background. At the same mean power output, not only did the ET athletes have drastically lower relative and absolute minute ventilation, respiratory rate, and heart rate, but they were performing at a much lower percentage of their $\dot{V}O_2$ peak. While efficiency was not calculated, it is obvious the ET group is mechanically more efficient.

Limitations

The major limitation of this study was the difficulty in performing the induced sputum procedure and the inherent variability in cell measurements in induced sputum samples. The variability of cell counts in induced sputum samples was a likely explanation for the limited statistical significance of some of the research findings.

Incomplete data sets for subjects unable to produce sputum at rest or following their moderate intensity time trial limited the sample size in these variables. Despite this limitation, cell composition of induced sputum of untrained and endurance trained subjects was similar to that previously found in controls and athletes (Bonsignore et al, 2001, Belda et al, 2000).

The timing of sputum induction was also a potential limitation of the present study. Morici et al. (2004) conducted sputum induction immediately post-exercise to avoid missing early changes after an exercise test of limited duration. However, Bonsignore et al. (2001) conducted sputum induction on average 202 ± 28min post-marathon. The timing of the sputum induction for this study provided a limited time frame of a late phase recovery after exercise. St Croix et al. (1999) found that during exercise the volume of expired NO (a noninvasive marker of airway inflammation) followed the changes in minute ventilation, with a rapid fall toward baseline in early recovery. However, Bonsignore et al. (2001) found that 3 hours after the marathon, exhaled NO in subjects was high, suggesting late changes in the time course of airway inflammation after exercise. The complex protocol of sputum induction and the known effects of repeated sputum inductions (Purokivi, 2000) limited the number of these maneuvers that can be done on the same day. According to experimental data, airway inflammation may still be present 24 hours after a dry air challenge in the dog (Omori, 1995). The timing of the sputum induction may contribute to the measured changes in airway cell composition and further investigation on this would be recommended.

In addition to the timing of sputum induction post-exercise, the duration of the exercise might also contribute to the changes in the airway cells. Morici et al. suggested

the duration of their all-out rowing test (200s ± 14s) was too short to cause neutrophil influx into the airways, as increased airway neutrophils in induced sputum were only found after very prolonged exercise such as a marathon race (Bonsignore et al, 2001). Helenius et al. (2000) found that short duration exercise in swimmers did not seem to induce significant airway inflammation. The timing of the exercise test for this study tried to replicate the duration of the all-out cycling test done by Hopkins et al (1997), which lasted 7-minutes. Due to the variability in research protocols involving acute exercise and airway cell composition, it is possible the duration of the time trial was too short to provide a maximal stimulus to the response of the inflammatory cells in airways.

It is also important to acknowledge that the training regimens of the endurance trained group were unable to be controlled. As a result, it can only be speculated that increased exercise neutrophilia at rest were secondary to exercise training. The difference in training programs between ET subjects may have impacted both baseline non-exercise control and exercise cell counts and added to the variability in their measurement.

Summary

In conclusion, the present research study showed several changes in sputum cell composition following intense exercise in both untrained and highly trained participants. The lack of a statistical change in red blood cells following exercise suggests that pulmonary hemorrhage did not occur in the majority of the trained or untrained subjects. Significantly higher neutrophils in the ET group at rest may be the result of the chronic training completed by the endurance athletes in this study. In addition, significant increases in bronchial epithelial cells in both groups following the maximal time trial suggests airway remodeling might be also be taking place at high minute ventilations.

However, significantly lower BEC in ET subjects following their moderate intensity time trial may provide evidence for a possible training adaptation. These findings are consistent with previous findings (Morici et al, 2004, Bonsignore et. al, 2001) however, since sputum induction is somewhat novel in its investigation involving exercise, additional research is recommended to further investigate the application of this technique. The role of exercise intensity and duration in modulating the type and degree of airway cell responses remains elusive.

3. 5 References

1. American Thoracic Society: Standardization of spirometry, 1994 update. *Am J Respir Crit Care Med.* 1995;152:1107–36)
2. Belda J, Leigh R, Parameswaran K, O'byrne P, Sears M, and Hargreave E. Induced sputum cell counts in healthy adults. *Am J Respir Crit Care Med* 161:475-478, 2000.
3. Bermon S. Airway inflammation and upper respiratory tract infection in athletes: is there a link? *Exerce Immunol Rev.* 13:6-14, 2007.
4. Bonsignore M, Morici G, Riccobono L, Insalaco G, Bonanno A, Profita M, Paterno A, Vassalle C, Mirabella A, and Vignola A. Airway inflammation in nonasthmatic amateur runners. *Am J Physiol Lung Cell Mol Physiol* 281: L668-676, 2001.
5. Bonsignore M, Morici G, Vignola A, Riccobono L, Bonanno A, Profita M, Abate P, Scichilone N, Amato G, Bellia V, and Bonsignore G. Increased airway inflammatory cells in endurance athletes: what do they mean? *Clinical Exp Allergy* 33:14-21, 2003.
6. Bradley B, Azzawi M, Jacobson M, Assoufi B. Collins JV, Irani AM, Schwartz LB, Durham SR, Jeffery PK, and Kay AB. Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. *J.Allergy Clin Immunol* 88: 661-674, 1991.

7. Chimenti L, Morici G, Paterno A, Bonanno A, Siena L, Licciardi A, Veca M, Guccione W, Macaluso F, Bonsignore G, and Bonsignore M. Endurance training damages small airway epithelium in mice. *Am J Respir Crit Care Med* 175: 442-449, 2007.
8. Crimi E, Pellegrino R, Smeraldi A, Brusasco V. Exercise-induced bronchodilation in natural and induced asthma effects on ventilatory response and performance. J Appl Physiol 92:2353-60, 2002.
9. Dempsey J & Wagner P. Exercise-induced arterial hypoxemia. J. Appl. Physiol. 87(6): 1997-2006, 1999.
10. Freed AN, Davis MS. Hyperventilation with dry air increases airway surface fluid osmolality in canine peripheral airways. *Am J Respir Crit Care Med* 159: 1101-1107, 1999.
11. Edwards MR, Hunte GS, Belzberg AS, Sheel AW, Worsley DF, and McKenzie DC. Alveolar epithelial integrity in athletes with exercise-induced hypoxemia. *J Appl Physiol* 89: 1537-1542, 2000.
12. Eldridge MW, Braun RK, Yoneda KY, and Walby WF. Effects of altitude and exercise on pulmonary capillary integrity: evidence for subclinical high altitude pulmonary edema. *J Appl Physiol* 100: 972-980, 2006.
13. Godin, G., & Shepard. R. A simple method to assess exercise behavior in the community. *Can J Appl Sport Sci* 10: 141-146, 1985.
14. Hanel B, Law I, and Mortensen J. Maximal rowing has an acute effect on the blood-gas barrier in elite athletes. *J Appl Physiol* 25: 25, 2003.

15. Helenius IJ, Rytila P, Metso T, Haahtela T, Venge P, and Tikkanen HO. Respiratory symptoms, bronchial responsiveness, and cellular characteristics of induced sputum in elite swimmers. *Allergy* 53: 346-352, 1998.

16. Hopkins SR, Schoene RB, Henderson WR, Spragg RG, Martin TR, and West JB. Intense exercise impairs the integrity of the pulmonary blood-gas barrier in elite athletes. *Am J Respir Crit Care Med* 155: 1090-1094, 1997.

17. Karjalainen E, Laitinen A, Sue-Chu M, Altraja A, Bjermer L, and Laitinen L. Evidence of airway inflammation and remodeling in ski athletes with and without bronchial hyperresponsiveness to methacholine. *Am J Respir Crit Care Med* 161: 2086-2091, 2000.

18. Laitinen LA, Laitinen A, and Haahtela T. Airway mucosal inflammation even in patients with newly diagnosed asthma. *Am Rev Respir Dis*. 147:697-704, 1993.

19. Levine SJ. Bronchial epithelial cell-cytokine interactions in airway inflammation. *J Investig Med* June; 43(3): 241-9, 1995.

20. MacKechnie JK, Leary WP, Noakes TD, Kallmeyer JC, MacSearraigh ET, and Olivier LR. Acute pulmonary oedema in two athletes during a 90-km running race. *S Afr Med J* 56: 261-265, 1979.

21. Morici G, Bonsignore M, Zangla D, Loredana Riccobono L, Profita M, Bonanno A, Paterno A, Giorgi R, Mirabella F, Chimenti L, Benigno A, Vignola A, Bellia V, Amato G, and Bonsignore G. Airway cell composition at rest and after an all-out test in competitive rowers. *Med Sci Sports Exercise* 36: 1723-1729, 2004.

22. Omori C, Schofield BH, Mitzner W, Freed AN. Hyperpnea with dry air causes time-dependent alterations in mucosal morphology and bronchovascular permeability. *J Appl Physiol*. Mar;78(3):1043-51, 1995.

23. Purokivi M, Randell J, Hirvonen MR, Tukiainen H. Reproducibility of measurements of exhaled NO, and cell count and cytokine concentrations in induced sputum. *Eur Respir J*. Aug;16(2):242-6, 2000.

24. St. Croix CM, Wetter TJ, Pegelow DF, Meyer KC, Dempsey JA. Assessment of nitric oxid formation during exercise. Am J Respir Crit Care Med. Apr: 159:1125-33, 1999.

25. Stickland MK, Welsh RC, Petersen SR, Tyberg JV, Anderson WD, Jones RL, Taylor DA, and Haykowsky MJ. Does fitness level modulate the cardiovascular hemodynaminc response to exercise? *J Appl Physiol* 100: 1895-1901, 2006.

26. Velden VH and Versnel HF. Bronchial epithelium: morphology, function and pathophysiology in asthma. Eur Cytokine Netw Dec; 9(4): 585-97, 1998.

27. Wagner PD, Gale GE, Moon RE, MmHge-Bueno JR, Stolp BW, and Saltzman HA. Pulmonary gas exchange in humans exercising at sea level and simulated altitude. *J Appl Physiol* 61: 260-270, 1986.

28. West JB. Invited Review: pulmonary capillary stress failure. *J Appl Physiol* 89: 2483-2489; discussion 2397, 2000.

29. West JB. Vulnerability of pulmonary capillaries during exercise. *Exerc Sport Sci Rev* 32: 24-30, 2004.

30. West JB, and Mathieu-Costello O. Stress failure of pulmonary capillaries: role in lung and heart disease. *Lancet* 340: 762-767, 1992.

31. Wetter TJ, Xiang Z, Sonetti DA, Haverkamp HC, Rice AJ, Abbasi Aa, Meyer KC, Dempsey JA. Role of lung inflammatory mediators as a cause of exercise-induced arterial hypoxemia in young athletes. *J Appl Physiol*. Jul;93(1):116-26, 2002.

Table 3.1. Subject characteristics and exercise performance measurements.

Characteristics and Performance Measurements	UT (n = 15)	ET (n =11)
Age (years)	21. 7 ± 3.3	25.4 ± 4.5*
Height (cm)	177.3 ± 9.0	180.2 ± 6.6
Weight (kg)	80.8 ± 16.5	71.7 ± 7.2
Godin Score (GLTEQ)	30.8 ± 15.0	83.5 ± 37.6*
FVC (L)	5.1 ± 0.9	5.41 ± 0.4
FEV_1 (L)	4.5 ± 0.6	4.9 ± 0.8
TLC (L)	7.1 ± 1.0	7.7 ± 1.1
DLCO ($ml \times mmHg^{-1} \times min^{-1}$)	36.4 ± 5.3	38.5 ± 5.4
DLCO adj V_A ($ml \times mmHg^{-1} \times min^{-1}$)	36.2 ± 5.8	38.5 ± 4.9
Absolute $\dot{V}O_2$ peak ($L \times min^{-1}$)	3.5 ± 0.5	4.5 ± 0.6*
Relative $\dot{V}O_2$ peak ($ml \times kg^{-1} \times min^{-1}$)	43.0 ± 3.7	62.6 ± 5.6*
PO max (watts)	310 ± 56	427 ± 45*
Peak HR ($b \times min^{-1}$)	187 ± 9.6	186 ± 8.5
Absolute $\dot{V}O_2$ @ VT ($L \times min^{-1}$)	2.2 ± 0.5	3.4 ± 0.5*
Relative $\dot{V}O_2$ @ VT ($ml \times kg^{-1} \times min^{-1}$)	27.2 ± 3.9	47.7 ± 4.7*
PO thresh (watts)	196 ± 38	307 ± 38*
HR @ VT ($b \times min^{-1}$)	152 ± 19	157 ± 11

Values are mean ± SD.
* = significantly different from UT group, $p < 0.05$.
UT = untrained.
ET = endurance trained.
PO= power output.
HR= heart rate.
VT= ventilatory threshold.

Table 3.2. Metabolic, cardio-respiratory, and performance results from the 5km time trial test.

	UT (n = 15)	ET-MOD (n= 9)	ET-HI (n = 11)
Absolute $\dot{V}O_2$ ($L \times min^{-1}$)	2.87 ± 0.61	2.87 ± 0.21 [b]	4.02 ± 0.77 [a]
Relative $\dot{V}O_2$ ($ml \times kg^{-1} \times min^{-1}$)	35.56 ± 1.98	40.34 ± 5.12 [ab]	55.95 ± 7.57 [a]
Absolute V_E ($L \times min^{-1}$)	114.07 ± 26.51	77.56 ± 19.56 [ab]	144.81 ± 24.07 [a]
Relative V_E ($L \times min^{-1} \times kg^{-1}$)	1.42 ± 0.19	1.10 ± 0.32 [ab]	2.02 ± 0.27 [a]
Absolute V_T (L)	2.64 ± 0.56	2.63 ± 0.48 [b]	3.11 ± 0.62
Relative V_T ($L \times kg^{-1}$)	0.03 ± 0.004	0.04 ± 0.005 [b]	0.04 ± 0.005 [a]
RR ($breaths \times min^{-1}$)	43 ± 7	31 ± 10 [ab]	47 ± 9
HR ($b \times min^{-1}$)	173 ± 10	146 ± 13 [ab]	175 ± 7
S_pO_2	96 ± 2	96 ± 1 [b]	93 ± 2 [a]
% of $\dot{V}O_2$ max	83 ± 0.1	65 ± 0.1 [ab]	89 ± 0.1
PO (watts)	210 ± 39	233 ± 3 [b]	343 ± 60 [a]
Speed ($km \times hr^{-1}$)	29 ± 4	32 ± 0.4 [ab]	38 ± 3 [a]
Cadence ($rev \times min^{-1}$)	83 ± 6	89 ± 8 [b]	96 ± 10 [a]
Total Time (sec)	603.50 ± 55.70	557.81 ± 7.07 [ab]	489.36 ± 40.22 [a]

Values are mean ± SD.
a = significantly different from UT-HI; $p<0.05$.
b = significantly different from ET-HI: $p<0.05$.
UT-HI = untrained all-out time trial.
ET-HI = endurance trained all-out time trial.
ET-MOD = endurance trained time trial at same absolute power output as UT-HI.
S_pO_2 = percentage oxygen saturation.

Table 3.3. Differential cell counts in sputum at rest and 2hr following the 5km TT.

	Group	Rest	2hr Post TT-MOD	2hr Post TT-HI
Eosinophils (%)	UT	0.14 ± 0.38		0.05 ± 0.10
	ET	0.15 ± 0.27	0.19 ± 0.50	0.25 ± 0.67
Neutrophils (%)	UT	27.18 ± 23.82		46.78 ± 26.29
	ET	48.63 ± 21.52[b]	44.94 ± 25.10	50.36 ± 24.9
Lymphocytes (%)	UT	2.32 ± 1.11		0.15 ± 0.23[ac]
	ET	2.43 ± 0.94	1.47 ± 0.78[acde]	0.18 ± 0.27[ac]
Alveolar Macrophages (%)	UT	65.39 ± 23.87		10.03 ± 10.68[ac]
	ET	45.85 ± 19.21	42.92 ± 19.86[ade]	8.98 ± 11.85[ac]
Bronchial Epithelial Cells (%)	UT	5.00 ± 4.38		42.95 ± 29.10[ac]
	ET	2.95 ± 4.44	10.47 ± 13.41[ade]	40.36 ± 24.71[ac]
Squamous Cells (%)	UT	5.21 ± 4.69		2.79 ± 4.73[a]
	ET	5.74 ± 5.97	9.39 ± 12.68	2.53 ± 3.48[a]
Red Blood Cells (%)	UT	0 ± 0		0.93 ± 2.57
	ET	0.1 ± 0.32	0.89 ± 1.36	1.00 ± 2.19
Total Cell Count	UT	0.47 ± 0.39		2.23 ± 3.82[a]
	ET	1.44 ± 1.45	1.18 ± 0.81	2.94 ± 2.98[a]

Values are mean ± SD.
a= TT-HI significantly different from rest, main effect between rest and exercise $p<0.05$.
b= significantly different from UT, group main effect, $p<0.05$.
c= significantly different from ET rest, interaction effect, $p<0.05$.
d= significantly different from UT TT-HI, interaction effect, $p<0.05$.
e= significantly different from ET TT-HI, interaction effect, $p< 0.05$.
TT-HI = All-out time trial.
TT-MOD = endurance trained time trial at same absolute power output as UT-HI.
REST- UT: N=11; ET: N=10.
TT-HI- UT: N=15; ET N=11.
TT-MOD- ET: N=9.

Table 3.4. Absolute sputum cell counts at rest and 2hr following exercise.

	Group	Rest	2hr Post	2hr Post
Eosinophils (/100 cells)	UT	0 ± 0		0 ± 0
	ET	0 ± 0.19	0 ± 0	0 ± 0.13
Neutrophils (/100 cells)	UT	24.24 ±28.13		51.75 ± 44.38
	ET	48.50 ± 36.25	42 ± 24.75	51.5 ± 39.25
Lymphocytes (/100 cells)	UT	2 ± 1.50		0 ± 0.25
	ET	2.63 ± 1.13	2 ±1.25	0 ± 0.38
Alveolar Macrophages (/100 cells)	UT	70.75 ± 29.75		8.25 ± 6.13
	ET	46.35 ± 34.13	41.75 ± 21.75	4.25 ±8.00
Bronchial Epithelial Cells (/100 cells)	UT	3.5 ± 4.38		39.5 ± 49
	ET	1.25 ± 2.25	4.25 ±8.25	36.75 ± 29.63
Red Blood Cells (/100 cells)	UT	0 ± 0		0 ± 0.50
	ET	0 ±0	0 ± 1	0 ± 0.50
Squamous Cells (/100 cells)	UT	3.4 ± 7.19		1.47 ± 2.66
	ET	4.075 ± 5.83	0.99 ± 22.71	0.5 ± 4.21
Total Cell Count	UT	0.47 ± 0.39		2.23 ± 3.82
	ET	1.44 ± 1.45	1.18 ± 0.81	2.94 ± 2.98

Values are median ± IQR.

REST- UT: N=11; ET: N=10.
TT-HI- UT: N=15; ET N=11.
TT-MOD- ET: N=9.

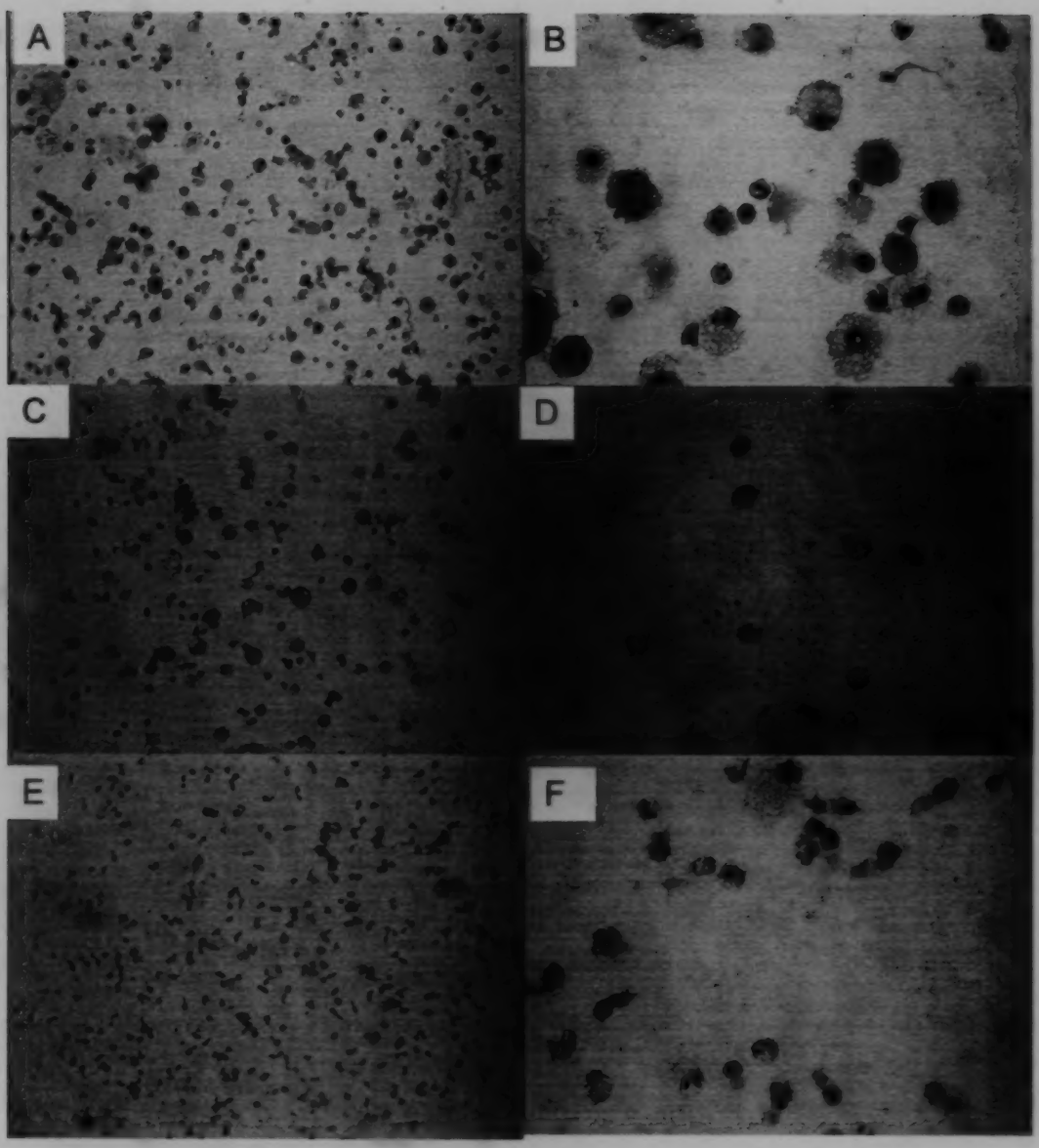


Figure 3.1. Representative picture of lung and airway cells in sputum (Diff Quick Stain) of one trained subject A. Rest 100x B. Rest 400X C. TT-MOD 100x D. TT-MOD 400x E. TT-HI 100x F. TT-HI 400x
Note: Increased proportion of bronchial epithelial cells (E) following all-out time trial.

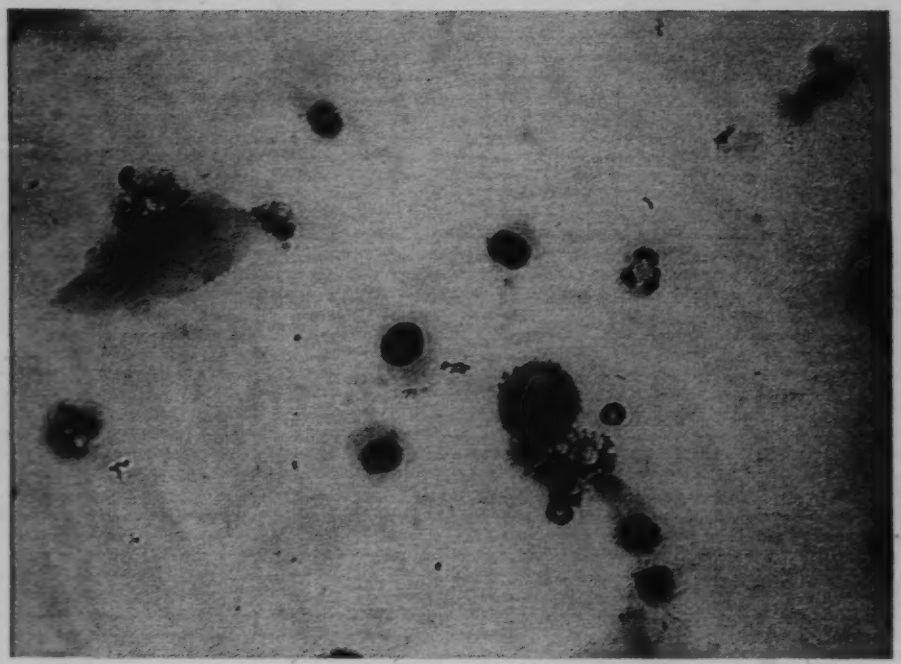


Figure 3.2. Diff Quick stain for red blood cells of one individual.

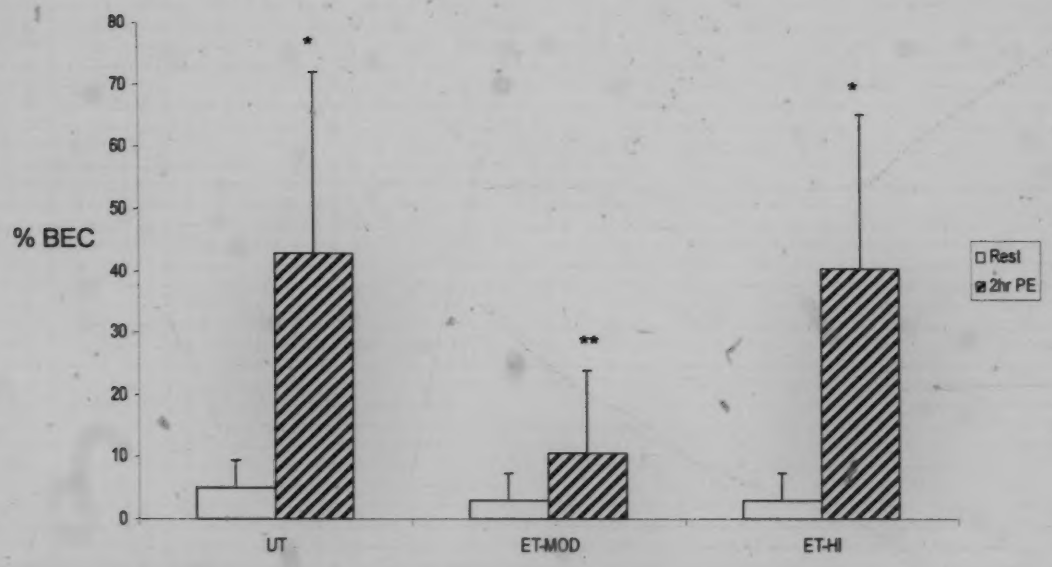
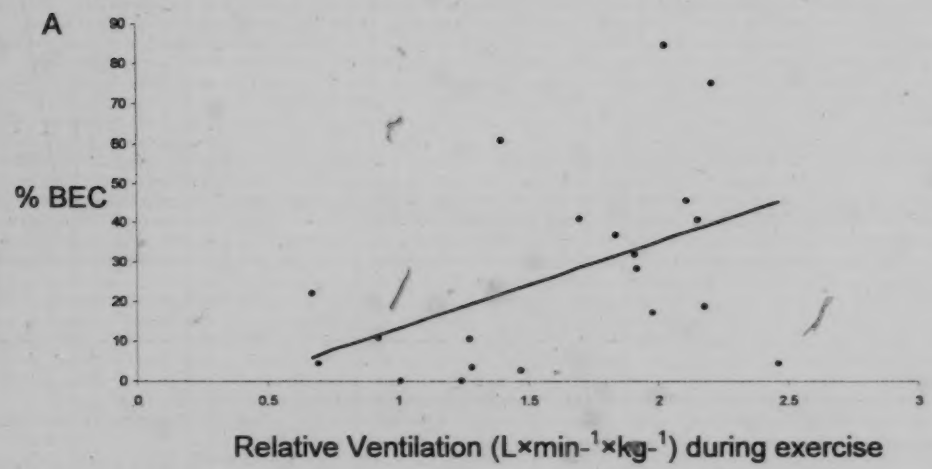


Figure 3.3. % Bronchial epithelial cells (BEC) at rest and 2 hrs following exercise in untrained (UT) and endurance trained (ET) participants.
ET-MOD = endurance trained time trial at same absolute power output as UT-HI.
ET-HI = endurance trained all-out time trial.
* = significantly different from rest, $P<0.05$.
**= significantly difference from UT and ET-HI, $P<0.05$.



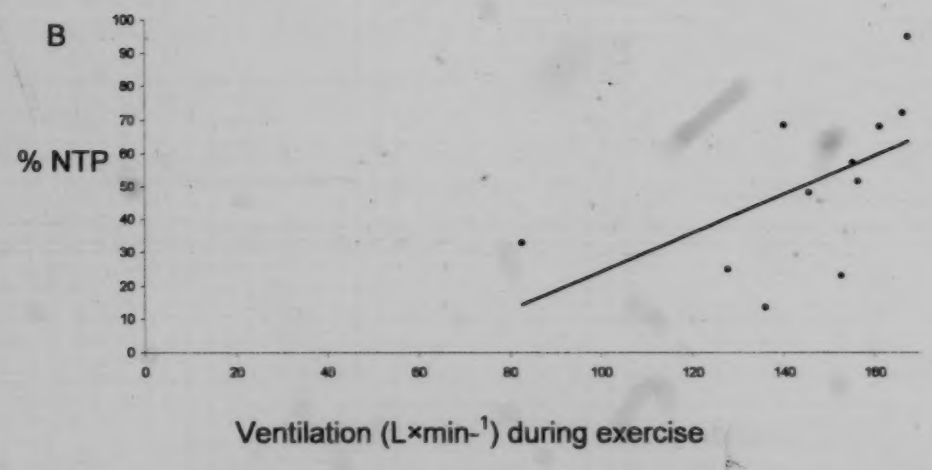


Figure 3.4. A. Relationship between relative ventilation and changes in % bronchial epithelial cells (BEC) of ET-HI and ET-MOD (r= 0.47, p ,0.05, N=20 . B. Relationship between ventilation and changes in % neutrophils (NTP) of ET-HI group (r= 0.39, p< 0.05, N=11).

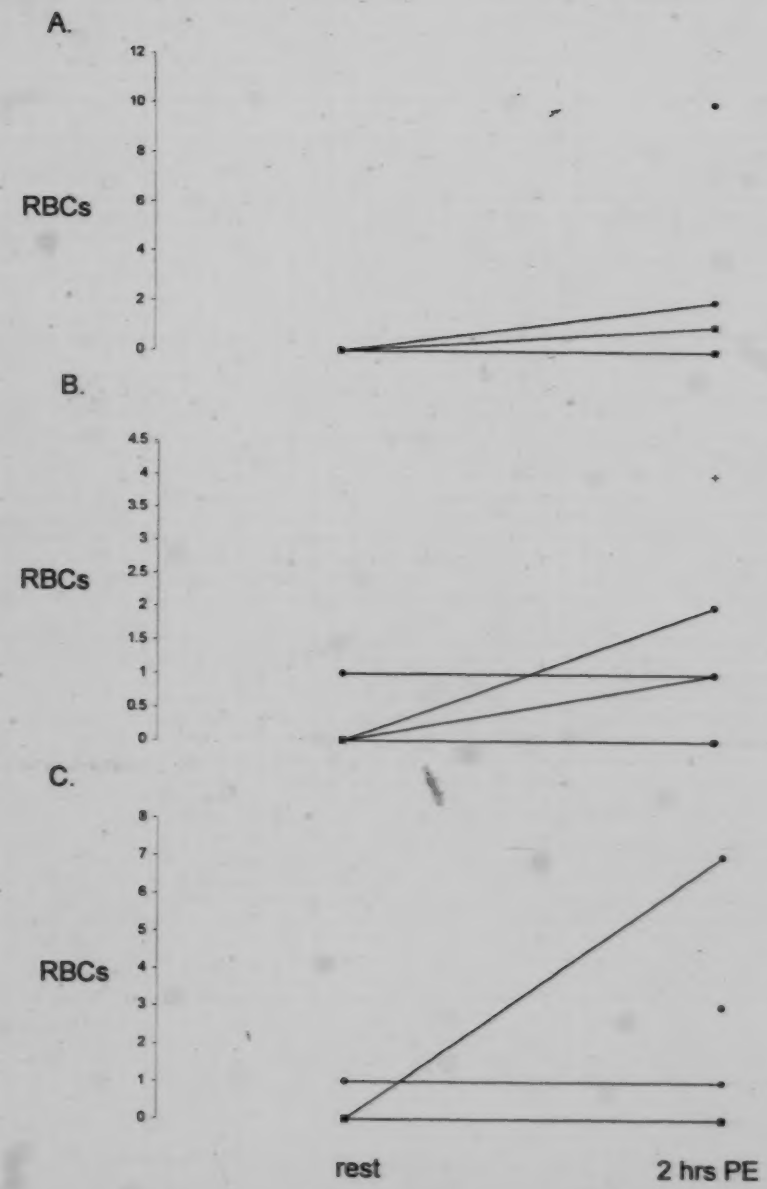


Figure 3.5. Individual red blood cell counts at rest and 2 hrs post-exercise (PE) for untrained (UT) and endurance trained (ET) participants.
A. UT B. ET-MOD C. ET-HI

Chapter 4

General Discussion and Future Recommendations

4.1 General Discussion

The existence of red blood cells in sputum samples following exercise is suggested to be indicative of an alteration in the permeability of the blood gas barrier that leads to damage of the blood gas barrier (Hopkins, 1997). Although there was a numerical increase in red blood cells 2 hours post-exercise in both the untrained and trained subjects of the present study, this increase did not reach statistical significance. The presence of the red blood cells in the sputum may have come from the bronchial tree or perhaps higher in the airways or throat. There were no red blood cells found at rest in any of the untrained subjects and four of those subjects produced red blood cells in their sputum following their all-out time trial. This may be an indication of lung damage following acute maximal exercise in those particular subjects. One endurance trained athlete had 1 red blood cell in his sputum sample at rest, three had red blood cells in their sputum following the all-out time trial (range = 1-7), and four had red blood cells following the moderate intensity time trial (range = 1-4). Interestingly, the ET subject that maintained the highest mean absolute and relative oxygen consumption during his all-out time trial and had the second highest $\dot{V}O_2$ peak also presented with the most number of red blood cells in his sputum sample. It is possible that exercise induced lung damage does not occur uniformly across all populations and may be that some athletes present with this condition while others do not for unknown reasons. Of the four athletes who produced red blood cells in their sputum following their moderate intensity time trial, one had red blood cells in his sputum at rest and two also produced red blood cells

in their sputum following their all-out time trial. The fourth subject was unable to produce a satisfactory sputum sample following his moderate intensity time trial. This suggests that perhaps the presence of red blood cells in sputum following intense or moderate exercise is also related to the specific person (individual reactorss vs. non-reactorss) and perhaps some athletes are more susceptible to lung damage than others.

It has been hypothesized that mechanism of exercise-induced pulmonary hemorrhage is increasing capillary pressures due to higher cardiac output in trained athletes (West and Costello, 1992). The presence of red blood cells in the sputum of the ET group 2 hours following the lower intensity time trial matched to the UT group may in fact contradict this latter assumption; that red blood cells are present because of increased pulmonary vascular pressures. Based on the metabolic and ventilatory data from the time trials, it was evident that at the same absolute power output, endurance trained athletes had significantly lower heart rate, absolute and relative $\dot{V}O_2$, absolute and relative VE, and respiratory rate compared to UT. Therefore, at the same intensity, endurance trained athletes were working at only 65% of their peak $\dot{V}O_2$. This is similar to findings by Stickland et al. (2006) where it was shown that pulmonary vascular pressure was lower in endurance trained athletes during exercise as compared to sedentary subjects. As such, it is possible the mechanism behind lung damage with exercise is not related to increased pulmonary artery pressures. However, pulmonary vascular pressures were not measured in this study and this inference requires further research.

It is also possible that the presence of red blood cells in sputum is not an indication of a disruption to the alveolar blood-gas barrier. Fahy et al. (1993) concluded

that induced sputum samples arise predominantly from airways rather than alveoli. It is possible that the blood in the sputum samples arose from raw and damaged airways that remained in a state of reconstruction due to heavy training and that the acute exercise bout was not solely responsible for the production of the red blood cells. As in other studies (Bonsignore et al, 2001, Morici et al, 2004), resting samples obtained before exercise samples in the endurance trained group were considered as mostly influenced by the chronic effects of habitual intense training. Samples obtained after intense exercise were considered to reflect the effects of acute exercise or the combined effects of chronic and acute exercise. In previous studies however, athletes did not do a moderate intensity exercise bout and sputum induction was only done at rest or following intense and/or prolonged exercise. In addition, the training regimen of the ET group was not strictly controlled and as such, it is possible outside factors contributed to the finding of red blood cells in sputum samples. Anecdotally, one of the ET subjects who had red blood cells in his sputum sample following his moderate intensity time trial admitted that he had an intense work-out the day prior.

An important finding of this study was the statistical increase in bronchial epithelial cells in endurance trained and untrained subjects following exercise that indicates a degree of airway deconstruction might be occurring. The mechanism of this exercise-induced change in both fitness groups following exercise can only be speculative. The correlation between exercise hyperventilation and increase inflammatory cell markers in sputum samples only suggests an association between the two variables and this may not be a causal relationship. However, it seems logical that high airflows generated during all-out exercise may cause epithelial damage through mechanical stress

on the airway wall (Morici et al, 2004). Bronchial epithelial cell derived cytokines may amplify ongoing inflammatory processes via the recruitment and activation of specific subsets of inflammatory cells, as well as by prolonging their survival in the airway microenvironment (Velden, 1998). Further investigation into the hyperosmolarity of airway surface lining or cooling-rewarming of the bronchial wall during intense exercise is required to establish the role of hyperventilation in airway inflammation.

The issue of exercise-induced changes in airway cells was initially addressed with regard to the pathogenesis of exercise induced bronchoconstriction (EIB). Exercise-related respiratory symptoms have been frequently shown in athletes, and a low exercise tolerance is common in asthmatic patients (Bonsignore et al, 2003). Subsequent studies on athletes did not find evidence that changes in airway cells may occur in athletes independent of symptoms or spirometric changes (Karjalainen et al, 2000), raising questions on the pathogenesis and possible consequences of chronic exercise training. Although bronchodilation occurs in healthy and asthmatic subjects during exercise (Crimi et al, 2002), bronchoconstriction can occur during early recovery after exercise. The main pathogenetic theory behind EIB is that exercise hyperventilation causes drying of the airways, thus increasing the osmolarity of the airway surface lining (Freed, 1995). This osmotic stimulus would make bronchial epithelial cells shrink and release inflammatory mediators, causing airway smooth muscle contraction (Anderson et al, 2000). Alternatively, EIB could reflect decreased airway caliber secondary to vascular engorgement at airway re-warming after exercise (McFadden, 1990). The subjects in the present study were negative for EIB and yet still provided evidence for airway remodeling following exercise as demonstrated by the significant change in bronchial

epithelial cells in both groups. Furthermore, ET athletes demonstrated significantly lower oxygen saturation during the all-out time trial than the UT group, with some athletes developing exercise-induced hypoxemia. However, none of the mentioned findings were correlated with exercise-related respiratory symptoms. As such, increased numbers of inflammatory cells in the airways of ET and UT groups were not necessarily associated with major clinical or functional alterations. To understand the physiological meaning of exercise-related airway changes in athletes and untrained subjects, further research is recommended.

4.2 Future Recommendations

The variable type and degree of airway inflammation observed following exercise indicates the need to extend observations to other populations and other sports. The role of exercise duration, intensity, and the timing of sputum induction after exercise is unclear. It is also uncertain whether habitual training causes long term changes, not only in elite athletes, but also in the large population of amateur athletes. Should chronic exercise be shown to modulate the intensity of airway inflammatory or bronchomotor responses to provocative stimuli, this finding could improve treatment and rehabilitation strategies in asthma or other clinical populations (Bonsignore et al, 2003). Finally, future studies should investigate the possible dose-response relationship between level of exercise-induced hyperventilation and airway changes.

4.3 References

1. Anderson SD, Daviskas E. The mechanism of exercise-induced asthma. *J Allergy Clin Immunol* 106: 453-459, 2000.
2. Bonsignore M, Morici G, Riccobono L, Insalaco G, Bonanno A, Profita M, Paterno A, Vassalle C, Mirabella A, and Vignola A. Airway inflammation in nonasthmatic amateur runners. *Am J Physiol Lung Cell Mol Physiol* 281: L668-676, 2001.
3. Bonsignore M, Morici G, Vignola A, Riccobono L, Bonanno A, Profita M, Abate P, Scichilone N, Amato G, Bellia V, and Bonsignore G. Increased airway inflammatory cells in endurance athletes: what do they mean? *Clinical Exp Allergy* 33:14-21, 2003.
4. Crimi E, Pellegrino R, Smeraldi A, Brusasco V. Exercise-induced bronchodilation in natural and induced asthma effects on ventilatory response and performance. J Appl Physiol; 92:2353-60, 2002.
5. Fahy JV, Liu J, Wong H, and Boushey HA. Cellular and biochemical analysis of induced sputum from asthmatic and from healthy subjects. *Am Rev Respir Dis* 147: 1126-1131, 1993.
6. Freed AN. Models and Mechanisms of exercise-induced asthma. *Eur Respir J* 8: 1770-1785, 1995.
7. Hopkins SR, Schoene RB, Henderson WR, Spragg RG, Martin TR, and West JB. Intense exercise impairs the integrity of the pulmonary blood-gas barrier in elite athletes. *Am J Respir Crit Care Med* 155: 1090-1094, 1997.

8. Karjalainen E, Laitinen A, Sue-Chu M, Altraja A, Bjermer L, and Laitinen L. Evidence of airway inflammation and remodeling in ski athletes with and without bronchial hyperresponsiveness to methacholine. *Am J Respir Crit Care Med* 161: 2086-2091, 2000.

9. McFadden ER. Hypothesis: exercise-induced asthma as a vascular phenomenon. *Lancet* 335: 880-883, 1990.

10. Morici G, Bonsignore M, Zangla D, Loredana Riccobono L, Profita M, Bonanno A, Paterno A, Giorgi R, Mirabella F, Chimenti L, Benigno A, Vignola A, Bellia V, Amato G, and Bonsignore G. Airway cell composition at rest and after an all-out test in competitive rowers. *Med Sci Sports Exercise* 36: 1723-1729, 2004.

11. Velden VH and Versnel HF. Bronchial epithelium: morphology, function and pathophysiology in asthma. Eur Cytokine Netw Dec; 9(4): 585-97, 1998.

12. West JB, and Mathieu-Costello O. Stress failure of pulmonary capillaries: role in lung and heart disease. *Lancet* 340: 762-767, 1992.

Appendix A

Participant Information

An Investigation of Pulmonary Damage and Airway Inflammation after Endurance Exercise in Individuals of Different Fitness Levels

Principal Investigator(s):
Miss Jori Hardin (780) 642-7189
Dr. Gordon Bell (780) 492 2018

Co-Investigator(s):
Dr. Michael Stickland (780) 407-7845
Dr. Dilini Vethanayagam (780) 407-1479

You are being asked to take part in a research study. This information and consent form explains this study. You will receive a copy of it. It may contain words that you do not understand. If you have any questions, or do not understand anything in this form, please ask the researcher to explain.

Introduction

We are interested in how the lungs work during exercise. Some research suggests that exercise may cause short-term inflammation/swelling in the lungs. This mild injury does not appear to be permanent, but many people report wheezing or coughing following hard exercise. We are interested in looking at what is causing this inflammation from exercise.

We will obtain samples from your airways using a technique call simple sputum induction. As will be detailed later, this technique is safe, and not invasive.
This study will investigate the effects of hard exercise on lung inflammation. We will also compare the responses from exercise based on fitness level.

Research Procedures

The research project is divided into two phases that we call 'screening' and 'experimental'. We are interested in studying the effects of exercise on individuals that have different fitness levels and who do not have any heart or lung conditions (including asthma, exercise-induced bronchoconstriction, and upper or lower respiratory tract infections). The results of the screening tests will confirm whether or not you are eligible to take part in the experimental portion of the study. If you are not eligible to participate, we will explain why and perhaps recommend that you consult your physician if necessary.

The plan for the study is shown below. We also have a preliminary 45 minute information session for all subjects to explain all the study procedures prior to signing a consent form.

Study Plan and Timeline

Screening Phase

Visit 1

Questionnaire
Standard Baseline and Lung Function Tests
(will take ~1hr)

Visit 2

Graded Exercise Test to VO_{2max}

Screening for exercise-induced bronchoconstriction (done along with exercise test) (~ 1.5hrs)

Experimental Phase

Visit 3

Simple Sputum Induction at Rest (~ 1hr)

Visit 4

Exercise Challenge #1:

5km All-out cycling time trial
-gas exchange and oxygen saturation will be measured
-sputum induction immediately and 2hrs post exercise
(~ 5 hrs)

Visit 5

Exercise Challenge #2:

We may ask you to perform a second time trial. This time trial will be paced a lower intensity than what you performed the week before.
-gas exchange and oxygen saturation will be measured
-sputum induction immediately and 2hrs post exercise

Visit 1:

During the first visit you will be asked to fill out a questionnaire that will help us find out if you have any health issues such as previously diagnosed lung/health problems. We will also measure how much air you can breathe in and out of your lungs in a special machine called a spirometer. This will be done in the Faculty of Physical Education and Recreation Exercise Physiology Laboratory. If there are no concerns after this, you will

be asked to have a more complete lung test in a lab (Pulmonary Function Lab) at the University of Alberta Hospital.

Visit 2:

The next part of the study is an exercise test to measure your fitness that will be done at the Exercise Physiology Lab at the University of Alberta. This test is called a maximum oxygen uptake (VO_2 max) test and will require you to exercise on a stationary bike that begins at an easy effort and then harder and harder every 2 minutes until you are very tired and cannot exercise any harder. At this point you will stop exercising on your own decision and the test will be over. The test usually lasts for about 12 to 15 minutes, with an additional 5 to 10 minutes of warm-up and cool-down exercise before and after. During the test, all the air that you breathe out will be collected in a special breathing device. Your heart rate will be measured with a heart rate monitor and how much oxygen is flowing through the blood vessels will be estimated using a small device clipped to your finger or on your earlobe.

Following the exercise test, we ask you to breath in and out as much air as you can to make sure you did not have any breathing issues (exercise induced bronchospasm or asthma). Beyond this, the main result from this test will be your aerobic fitness level (VO_{2max}). This information helps us to make sure that your aerobic fitness level is at a level that we need for the study and if it is not, then unfortunately, you will not be able to be a participant in the study.

Visit 3:

If you are willing and able to join our study, you will be asked to come back a few days later and do some more testing. At this time, we will ask you to gently breathe in a salt-water mist (saline solution) through a hand held device. This will happen 3 different times for 7 minutes at a time. After each time you breathe the mist for 7 minutes, we will ask you to force yourself to cough and spit 2 or 3 times into a cup. Then we will test how much air you can breathe into and out of your lungs the same way as you did before. This happens 3 times.

Important note: You may be asked to perform Visit 4 first and then Visit 3. The order will be randomly assigned by the researcher.

Visit 4:

The next visit is about a week later and this time you will come to the Exercise Physiology Lab for a different type of exercise test. This test will require you to warm up on a stationary bike and then when you are ready we will ask you to cycle 5 km as fast as you can. This should take you about 10 minutes depending on the person. Again, during

the test, you will breathe through a special breathing device that allows us to measure how much oxygen you are using during the test. Heart rate and the oxygen content of your blood using the finger or earlobe clip will also be done this same way.

Immediately and 2 hours after this exercise test, you will do the same coughing and spitting test as previously mentioned. This entire testing will take approximately 5 hours.

Visit 5:

We may ask you to perform a second time trial. This time trial will be paced a lower intensity than what you performed during Visit 4. During the test (like the other), you will breathe through a special breathing device that allows us to measure how much oxygen is being used. Heart rate and blood oxygen levels will be monitored every minute. Immediately after and 2 hours after each 5 km exercise test, you will do the same coughing and spitting test as previously mentioned.

Important note: The order of Visit 4 and Visit 5 (if you are asked to come in for Visit 5) will be randomly determined by the researcher. We ask that you not participate in exercise the day before each test.

Time Commitment

It is estimated that the total time required to participate in this study will be about 10 hours during 3 different sessions if you are in the untrained group spread over 3 weeks and 15 hours during 4 different sessions spread over 4 weeks if you are in the trained group (see study plan diagram above).

Risks

The exercise challenges present minimal risk to healthy, physically active adults. The risks are even lower in well-trained athletes that do this type of exercise on a regular basis.

However, there is always some health risk associated with maximal exercise even for athletes. The maximal oxygen consumption test requires maximal effort in order to go to exhaustion. There may be some health risk with this type of exercise. During and following the test, it is possible to experience symptoms such as abnormal blood pressure, fainting, light-headedness, muscle cramps or strain, nausea and, in very rare cases (0.5 per 10,000 in testing facilities such as exercise laboratories, hospitals and physician offices), heart rhythm disturbances or heart attack. While serious risk to healthy participants is highly unlikely, they must be acknowledged and participants willingly assume the risks associated with very hard exercise. The exercise test will be administered by qualified personnel under the supervision of Dr. Gordon Bell. Personnel are trained to handle identifiable risks and emergencies, and have certification in CPR. Certifications can be produced if requested. In addition, an emergency protocol has been established for our laboratory should unforeseen circumstances arise.

During all the experimental parts of the study, an emergency response for medical reasons is initiated by phoning 2-5555 when in the Exercise Physiology Lab. Highly qualified medical personnel (physicians, nurses, and technicians) with special training will be available/on call and can be reached via pager for any testing in the U of A hospital.
You can stop the exercise tests at any time. The researchers may terminate the exercise protocols if they are concerned about your safety. You are asked to tell the researchers of any symptoms such as pain, illness, or unusual fatigue experienced during any part of the study.

The procedures described are considered less risky with healthy patients and to date, there have been no reports of any serious adverse incidents using these techniques with healthy subjects. Spirometry may occasionally give a transient headache. Breathing the saline mist required to obtain a sputum sample (phlegm sample) can occasionally result in a sore throat, coughing or wheezing, but can be relieved with a lozenge. Rarely, does any healthy person react badly to the sputum procedures.

Benefits

There may be no direct benefits from participation however it is expected that the information that you receive from participation will be of interest and value to you. The researchers will be pleased to discuss personal fitness results and answer questions during the study. You will be given a complete set of your personal results as soon as possible after the full study is completed. The results of the research will be of value to the field of applied exercise physiology.

Confidentiality of Information

All information collected in the study will be maintained in a confidential fashion. Individual data will be assigned a code designation so that personal identification is not possible. The data will be stored in a secure location accessible only to the researchers. If you withdraw from the study, your information will be removed from the study on request. The coded results will be used for scientific research only. The research information is stored for 7 years after the study is completed, and the Health and Research Ethics Board might have access to the data in case of an audit during this time. Otherwise, after the 7 year period, it will be destroyed.

Questions or Concerns

If you have questions about your participation in the study, we encourage you to contact one of the researchers. If you have other concerns about this research you may contact Dr. Marcel Bouffard, Associate Dean for Research (492-5910) in the Faculty of Physical Education and Recreation. His office has no direct affiliation with this study.

INFORMED CONSENT FORM

To be completed by the research subject — Yes No

1. Have you been told you are being asked to participate in a research study?
2. Have you received a copy of the attached information sheet?
3. Have you read and understand the Informed Consent Package including any benefits or risks involved in taking part in this research study?
4. Have you had an opportunity to ask questions and discuss this study with study personnel?
5. Have you been told and understand that participation in this study is strictly voluntary and that you may withdraw from the study at any time without having to give a reason?
6. Has the issue of confidentiality of your study records been explained to you?
7. Have you been told that your family doctor may be informed of your participation in this study?
8. This study was explained to me by:

_____
Name of Person Explaining Study

Yes No

I, _____ agree to take part in this study
Printed Name of Research Subject

_____ _____
Signature of Research Subject Date

_____ _____
Signature of Witness Date

To be completed by study personnel

_____ _____
Signature of Study Director Date

Appendix B Pulmonary Function Questionnaire

Name:

	YES	NO
1. Have you ever received treatment for asthma?		
2. Are you currently taking asthma medication?		
3. Are you currently taking allergy shots?		
4. Are you currently taking antihistamine tablets?		
5. Are you currently taking decongestants?		
6. Have you ever received treatment for eczema?		
7. Do you suffer from allergies?		
8. Have you ever missed school or work because of chest tightness/cough/wheezing or prolonged shortness of breath?		
9. Do you smoke tobacco?		
10. Do you ever have chest tightness?		
11. When you exercise do you often have chest tightness?		
12. Do you cough or wheeze after strenuous exercise?		
13. Do you ever experience "Locker Room" coughing after strenuous exercise?		
14. Do you suffer from frequent chest infections during the competitive seasons?		
15. Do you become easily "winded" during pre-season conditioning drills, or out of shape by mid-season?		
16. Have you ever been diagnosed by a physician for lung disease, heart disease, heart murmur?		
17. Have you had any viral (upper or lower airway) infection within the last four weeks?		

Appendix C

Godin Leisure-Time Exercise Questionnaire

1. During a typical **7-Day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your free time (write on each line the appropriate number).
In Times Per Week

a) STRENUOUS EXERCISE
(HEART BEATS RAPIDLY) _____
(e.g., running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling)

b) MODERATE EXERCISE
(NOT EXHAUSTING) _____
(e.g., fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)

c) MILD EXERCISE
(MINIMAL EFFORT) _____
(e.g., yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking)

2. During a typical **7-Day period** (a week), in your leisure time, how often do you engage in any regular activity **long enough to work up a sweat** (heart beats rapidly)?

OFTEN SOMETIMES NEVER/RARELY
1. ☐ 2. ☐ 3. ☐

Appendix D: Correlation Matrices for All-out time trial data and ventilatory and Metabolic variables

	VO2	Rel VO2	RER	VE	VE/ KG	RR	VT	VT/KG	HR	SaO2	% VO2	Eos	Neut	Lymph	Mac	Epi	Squames	RBC
VO2	1.000	.815	-.162	.886	.673	.191	.841	.627	.164	-.435	.788	.004	.082	.187	-.150	-.027	-.034	.001
Rel VO2	.815	1.000	-.164	.652	.871	.208	.579	.818	.166	-.536	.555	.086	.115	.043	-.288	-.011	-.061	.037
RER	-.162	-.164	1.000	.159	.176	.225	.027	.022	.142	-.063	.183	.428	-.112	-.260	-.346	.213	.218	.135
VE	.886	.652	.159	1.000	.744	.475	.747	.463	.140	-.365	.857	.138	.026	-.033	-.329	.085	-.076	-.031
VE/ KG	.673	.871	.176	.744	1.000	.530	.436	.682	.139	-.452	.602	.259	.096	-.181	-.473	.067	-.108	.018
RR	.191	.208	.225	.475	.530	1.000	-.220	-.250	.045	-.138	.381	.418	-.141	-.301	-.209	.198	-.352	-.148
VT	.841	.579	.027	.747	.436	-.220	1.000	.718	.166	-.317	.653	-.130	.138	.212	-.169	-.072	.183	.095
VT/KG	.627	.818	.022	.463	.682	-.250	.718	1.000	.172	-.428	.380	-.053	.186	.068	-.337	-.060	.174	.140
HR	.164	.166	.142	.140	.139	.045	.166	.172	1.000	-.412	.250	.031	-.038	-.187	-.063	.058	.608	.414
SaO2	-.435	-.536	-.063	-.365	-.452	-.138	-.317	-.428	-.412	1.000	-.249	-.204	.347	.135	.334	-.435	-.031	-.118
% VO2	.788	.555	.183	.857	.602	.381	.653	.380	.250	-.249	1.000	.092	.028	-.088	-.293	.072	.143	.183
Eos	.004	.086	.428	.138	.259	.418	-.130	-.053	.031	-.204	.092	1.000	.028	-.112	-.129	-4.767E-4	-.065	-.067
Neut	.082	.115	-.112	.026	.096	-.141	.138	.186	-.038	.347	.028	.028	1.000	.125	.010	-.942	.175	.087
Lymph	.187	.043	-.260	-.033	-.181	-.301	.212	.068	-.187	.135	-.088	-.112	.125	1.000	.677	-.349	-.017	-.140
Mac	-.150	-.288	-.346	-.329	-.473	-.209	-.169	-.337	-.063	.334	-.293	-.129	.010	.677	1.000	-.346	-.072	.017
Epi	-.027	-.011	.213	.085	.067	.198	-.072	-.060	.058	-.435	.072	-4.767E-4	-.942	-.349	-.346	1.000	-.140	-.086
Squames	-.034	-.061	.218	-.076	-.108	-.352	.183	.174	.608	-.031	.143	-.065	.175	-.017	-.072	-.140	1.000	.734
RBC	.001	.037	.135	-.031	.018	-.148	.095	.140	.414	-.118	.183	-.067	.087	-.140	.017	-.086	.734	1.000

Pulmonary Function Testing variables

	FVC	FEV1	TLC	DLCO	DLCO/VA	Eos	Neut	Lymph	Mac	Epi	Squames	RBC
FVC	1.000	.936	.949	.751	.593	-.265	.051	.108	-.003	-.042	-.182	.108
FEV1	.936	1.000	.885	.806	.662	-.144	.032	.069	-.108	.013	-.253	.036
TLC	.949	.885	1.000	.671	.499	-.230	.070	.235	.031	-.074	-.052	.098
DLCO	.751	.806	.671	1.000	.938	.012	-.289	-.074	-.125	.314	-.136	.074
DLCO/VA	.593	.662	.499	.938	1.000	.131	-.365	-.111	-.063	.360	-.196	.080
Eos	-.265	-.144	-.230	.012	.131	1.000	.111	-.143	-.112	-.084	-.162	-.121
Neut	.051	.032	.070	-.289	-.365	.111	1.000	.197	-.015	-.932	-.129	.236
Lymph	.108	.069	.235	-.074	-.111	-.143	.197	1.000	.804	-.479	.469	.507
Mac	-.003	-.108	.031	-.125	-.063	-.112	-.015	.804	1.000	-.348	.505	.395
Epi	-.042	.013	-.074	.314	.360	-.084	-.932	-.479	-.348	1.000	-.062	-.365
Squames	-.182	-.253	-.052	-.136	-.196	-.162	-.129	.469	.505	-.062	1.000	.101
RBC	.108	.036	.098	.074	.080	-.121	.236	.507	.395	-.365	.101	1.000

Appendix E: EIB Screening- FEV_1 at baseline, 1 min, 3min, 5min, 10min and 15min following GXT

Subject	Baseline	1	3	5	10	15
1	4.059	4.144	4.113	4.028	4.111	4.28
2	5.02	5.427	5.738	5.262	5.07	5.091
3	5.957	6.102	6.069	6.037	5.78	5.718
4	4.935	5.128	4.882	4.971	4.677	4.678
5	4.375	4.67	4.523	4.554	4.398	4.49
6	4.519	4.616	4.882	4.718	4.676	4.649
7	4.988	5.379	5.691	5.934	5.862	5.511
8	4.04		3.741	3.751	4.141	4.563
9	4.193	4.741	4.204	4.148	4.031	4.083
10	5.069	5.681	5.488	5.44	5.366	5.32
11	3.06	3.393	3.174	3.232	3.107	3.004
12	4.933	4.895	4.877	4.99	4.796	4.871
13	4.83			4.528	4.542	4.546
14	4.802	5.021	4.729	4.527	4.774	4.966
15	3.319	3.138	3.234	3.238	3.397	3.465
1	3.82	3.939	3.861	3.82	3.66	3.79
2	5.718	6.293	6.147	5.887	5.83	5.825
3	4.42	4.504	4.432	4.415	4.371	4.546
4	5.206	5.059	5.345	4.974	4.869	5.132
5	4.735	4.835	5.124	5.026	5.06	4.729
6	4.612	4.595	4.797	4.219	4.508	4.656
7	3.833	3.784	3.887	3.569	3.795	3.965
8	5.943	6.442	6.624	6.802	6.925	7.119
9	4.228	4.478	4.346	4.32	4.31	4.491
10	5.555	4.917	5.517	5.6	5.497	5.168
11	5.536	5.634	5.593	5.524	5.526	5.448

Appendix F: Reliability of Cell Counts

	bronc.epi /100	bronc.epi /100	mean	SD	CV
103A	1	0	0.5	0.707107	1.414214
106B-H	28	25	26.5	2.12132	0.08005
4A	3	1	2	1.414214	0.707107
15B	37	36	36.5	0.707107	0.019373
group mean	17.25	15.5	16.375	1.237437	0.555186
	neuts /100	neuts /100	mean	SD	CV
103A	28	26	27	1.414214	0.052378
106B-H	67	71	69	2.828427	0.040992
4A	26	25	25.5	0.707107	0.02773
15B	53	55	54	1.414214	0.026189
group mean	43.5	44.25	43.875	1.59099	0.036822
	macs /100	macs /100	mean	SD	CV
103A	69	72	70.5	2.12132	0.03009
106B-H	5	4	4.5	0.707107	0.157135
4A	69	72	70.5	2.12132	0.03009
15B	10	9	9.5	0.707107	0.074432
group mean	38.25	39.25	38.75	1.414214	0.072937